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Pathapelly Raja Shekhar Department of Silviculture and Agroforestry, FCRI, Mulugu, Telangana, India

Mhaiskar Priya Rajendra

Assistant Professor, Department of Forest Ecology and Climate Science, Forest College and Research Institute, Mulugu, Siddipet, Telangana, India

Milkuri Chiranjeeva Reddy

Department of Silviculture and Agroforestry, FCRI, Mulugu, Telangana, India

Reeja Sundaram

Department of Tree Breeding and Improvement, FCRI, Mulugu, Telangana, India

Sreedhar Bodiga

Department of Basic and Social Sciences, FCRI, Mulugu, Telangana, India

Ooha M

Department of Silviculture and Agroforestry, FCRI, Mulugu, Telangana, India

Ravula Rohith Department of Silviculture and Agroforestry, FCRI, Mulugu, Telangana, India

Indu Kale Department of Forestry, IGKV, Raipur, Chhattisgarh, India

Bhargavi CH Department of Forestry, IGKV, Raipur, Chhattisgarh, India

Corresponding Author: Mhaiskar Priya Rajendra

Assistant Professor, Department of Forest Ecology and Climate Science, Forest College and Research Institute, Mulugu, Siddipet, Telangana, India

Effect of pre-sowing treatments and growth hormones on germination and propagation of *Terminalia chebula* Retz

Pathapelly Raja Shekhar, Mhaiskar Priya Rajendra, Milkuri Chiranjeeva Reddy, Reeja Sundaram, Sreedhar Bodiga, Ooha M, Ravula Rohith, Indu Kale and Bhargavi CH

Abstract

Terminalia chebula is an important medicinal tree species in Asian countries. It is considered as the "King of Medicines "and is always listed at the top in Ayurvedic Materia Medica due to its extraordinary power of healing. However, Large scale planting program of the species is often difficult due to nonavailability of quality planting material, which is impacted by low seed germination & longer germination period. The present Investigation was conducted in the nursery unit of the Department of Silviculture and Agroforestry, Forest College and Research Institute, Mulugu, Telangana during 2021-2022. The experiment was laid out in a completely randomized design with ten treatments and three replications. Ninety seeds of *Terminalia chebula* were used for each treatment and these seeds were sown on seedbed of 10m x 1m with potting media of soil, sand, and FYM in 2:1: 1. Among all the treatments, seeds of Terminalia chebula soaked in water treatment (room temperature) for 24 hours (T₃) was shown significantly superior performance with respect to germination percentage (78.88%) and Germination Energy (50.0%). However, maximum survival percentage (98.07%), Shorter Germination period (19 days), maximum root length (11.20 cm), shoot length (14.22 cm), Number of Leaves (7.81) and Collar diameter (1.93 cm), was observed in GA3 500 ppm for 24 hours (T9). While, Dry weight of Root (1.30 g) and Shoot (1.75 g) was influenced by GA₃ 300 ppm (T₈) whereas shoot: root ratio was maximum (2.80) in 50% Conc. H₂SO₄ for 2 minutes (T4). Therefore, pre-sowing treatment of seeds soaked in water treatment (room temperature) for 24 hours was more effective in germination and GA3 of 500 ppm for 24 hours was found to give better growth of quality seedlings of Terminalia chebula in the nursery.

Keywords: Pre-sowing treatments, seed germination, seedling growth, Terminalia chebula

Introduction

Terminalia is a large genus of predominantly deciduous trees and a few climbers. The genus is named after the Latin phrase 'Terminus,' which relates to how near the leaves are to the stalk tips. It has more than 200 species, making it the most diverse in South East Asia (Fahmy *et al* 2015) ^[8]. *Terminalia chebula* belonging to Combretaceae family, is a common medicinal tree species used for a number of purposes in the Indian subcontinent. It is generally known as Chebulic myrobalan and also known as "Harad" in Hindi, "Allale" in Kannada, and "Karrakya" in Telugu (Sharma *et al.*, 1995) ^[34]. *Terminalia chebula* is a medium- to large-sized tree native to Asia's tropical and subtropical regions, including China and Tibet (Kannan 2009) ^[15]. In Tibet, *Terminalia chebula* is called as 'The king of medicines' (Bag *et al.*, 2013; Pellati *et al.*, 2013) ^[2], can grow in a wide range of soil conditions, including clay and sandy soils. The trees can grow to be around 20 meters tall and survive in areas with annual precipitation ranging from 900 to 4000 mm and temperatures ranging from 15 to 47.5 degrees Celsius. It grows in deciduous woods in Himachal Pradesh, Tamil Nadu, Kerala, Karnataka, Uttar Pradesh, Andhra Pradesh and West Bengal in India. (Gowda *et al.*, 2011) ^[10].

Terminalia chebula is a well-known traditional medicine used not just in India, but also throughout Asia and Africa (Baliga *et al.* 2012). It is one of the essential constituents of the ayurvedic drug "Triphala," along with *Terminalia bellerica* and *Emblica officinalis* (Naik *et al.*, 2003) ^[22]. The ripe fruits of *Terminalia chebula* are purgative and are used in pharmaceuticals such as laxatives, stomachics, and tonics (Krishnan 1998) ^[16] and used to cure piles, dropsy, diarrhea, headaches, dyspepsia, and ascites (Chang and Lin. 2010) ^[5] and also used in conjunction with cholesterol-lowering medicines (Usha 2007) ^[39].

This plant is also utilized as a food source for animals (Kumar and Bhatt 2006) ^[17]. It contains nutrients such as vitamin C, protein, amino acids, and minerals (Mahesh *et al.*, 2007) ^[20].

Despite of its various benefits, *Terminalia chebula* plants are threatened, which is one of the main reasons for their population decline across the world. *Terminalia chebula* seeds have a low germination rate in the field attributed to the prevalence of a hard mesocarp in the seed, a thick shell (endocarp), and poor kernel development (Troup 1921)^[37]. The fruit of *Terminalia chebula*, which is technically a drupe with a hard endocarp, must germinate over an extended period of time in ideal soil conditions. Rats, squirrels, and rodents devour the fruits which are spread out in the natural population, leading to poor natural regeneration of *Terminalia chebula* (Singh *et al.*, 2003)^[35]. As a result, effective presowing seed treatments can accelerate the germination. (Hossain *et al.*, 2013)^[14].

Pre-sowing treatment is a seed treatment that refers to the treating of seeds with any chemical, physical, or biological means before sowing in order to accelerate seed germination. (Umarani *et al.*, 2004) ^[38]. Seed treatments are practiced to ensure faster and uniform germination of seeds. As a result, much research has been conducted to create effective seed treatments for removing dormancy and breaking the hard seed coat of various species in order to assure faster and more effective germination (Nasim *et al.*, 1996) ^[23]. Therefore, the study has been conducted to improve the germination parameters and seedling growth performances at the nursery stages by giving varied treatments to the seeds to overcome the poor germination constraints in this commercially important tree species.

Material and Methods

Planting material and Experimental Details

The mature seeds of *Terminalia chebula* were collected from Khammam forest area, Telangana in September 2021. The study was conducted in the field Nursery of Department of Silviculture and Agroforestry, Forest College and Research Institute, Mulugu, Siddipet, Telangana during 2021-2022. The experimental site is located at 17°.728544 N latitude to 78°.63296 E longitude. The region is dominated by the red shallow gravelly soils, red clayey soils, deep calcareous and colluvial soils. The mean annual temperature ranges from 18 °C to 42 °C and average rainfall of this area is 905 mm. The seeds are sown by dibbling method in the nursery beds of the size 10 m x 1m with soil, sand and FYM as potting mixture in the ratio of 2:1:1 for the primary beds. The experimental design was laid out using Completely Randomized Design (CRD) with nine treatments and three replications each. 90 seeds were sown for each treatment cumulatively 900 seeds for all the treatments of the species. And the results are interpreted using One-way analysis of variance (ANOVA) -Completely Randomized Design (Panse and Sukatme, 1995) [26]

T ₁	water Treatment for 12 hours (Room Temperature)
T ₂	water Treatment for 24 hours (Room Temperature)
T ₃	water Treatment for 48 hours (Room Temperature)
T ₄	50% H ₂ SO ₄ for 2 minutes
T5	50% H ₂ SO ₄ for 5 minutes
T ₆	50% H ₂ SO ₄ for 10 minutes
T ₇	100ppm GA ₃ for 24 hours
T ₈	300ppm GA ₃ for 24 hours
T 9	500ppm GA ₃ for 24 hours
T ₁₀	Control

Germination attributes

Germination parameters are recorded from the day of commencement of germination. The parameters investigated in the present study are Germination Percentage (Maguire 1962) ^[19], Germination Energy (Ruan *et al.* 2002) ^[32], Germination Period and Survival Percentage (Sajana 2016) ^[33] (Table 1)

Growth attributes

Growth parameters like Root length, shoot length, Number of leaves, Collar diameter were recorded at 45 days interval up to 90 days and dry weight of root, dry weight of shoot and shoot: root ratio after 90 days. Digital caliper was used to measure the collar diameter (Table 2)

Results and discussion

Germination parameters of *Terminalia chebula*

Data presented in Table 2 showed that significant differences at the 5% level among different treatments applied. The observations showed that the maximum germination percentage (78.8%) was observed in seeds were soaked in water treatment (room temperature) for 48 hours (T_3) and the minimum germination percentage (33.3 %) was recorded in water treatment (room temperature) for 12 hours (T_1) . Improvement in germination of seeds might be due to the stimulating effect of imbibition's on seed germination caused by increased water absorbing capacity and killing pathogens (Cho and Lee 2018). The results are in accordance with the work done by Hossain et al., (2005)^[13] found that seeds of chebula showed maximum germination Terminalia percentage (66.7%) when subjected to soaking in cold water for 48 hours and also Kumar (2016) [18] on Terminalia bellirica (Bahera).

However, the maximum germination energy (50%) was recorded in seeds soaked in water treatment (room temperature) for 48 hours (T₃) and the minimum germination energy (20%) was recorded in water treatment (room temperature) for 12 hours (T₁). The results are in agreement with those obtained by Hossain *et al.*, (2005) ^[13] who stated that highest germination energy (58.9) in *Terminalia chebula* was observed when seeds were soaked in cold water for 48 hours.

The shorter germination period for (19 days) was noticed in seeds treated with GA₃ of 500 ppm for 24 hours (T₉) and the longer germination period (28 days) was observed in control (T_{10}) . The maximum survival percentage (98.07%) was observed in seeds treated with GA₃ of 500 ppm for 24 hours (T_9) whereas the minimum survival percentage (76.66%) was recorded in seeds soaked in water treatment (room temperature) for 12 hours (T_1) . This could be due to the weakening of the seed coat for gaseous and moisture exchange, as well as the availability of food material essential for early and better germination under this treatment. Early germination may have resulted in faster and stronger root development, which may have aided in the development of stem and leaves in these seedlings, resulting in a higher survival percentage. This obtained results goes in line with the findings of Manekar (2011) [21] discovered that Emblica officinalis seeds treated with GA₃ 200 ppm for 24 hours have resulted in the highest (92.73%) seedling survival percentage and also Dilip et al., (2017)^[7] in Rangpur Lime; Palepad et al., (2017)^[25] in custard apple (Annona squamosa); Barche et al., (2010)^[4] in papaya (*Carica papaya*).

Treatment Details	GP (%)	SP (%)	GE (%)	G.PE (days)
T1	33.33	76.66	20.00	25
T ₂	48.88	84.09	33.33	23
T3	78.88	85.91	50.01	21
T_4	53.33	87.50	34.44	20
T5	61.11	81.81	38.88	22
T ₆	56.66	82.35	37.77	23
T7	65.55	83.05	41.11	24
T ₈	70.00	93.96	46.66	20
T9	57.77	98.07	43.33	19
T ₁₀	51.11	82.60	31.11	28
S.Em±	0.541	0.293	1.378	1.906
CD (@5%)	1.608	N/S	4.095	5.663

Table 1: Effect of different pre-sowing treatments on Germination parameters of *Terminalia chebula*

*GP - Germination percentage * GE - Germination energy

* G.PE - Germination period * SP- Survival percentage

* SEm± - Standard error mean * CD (@5%) – Critical difference at 5 % level of significance * N/S= Non –Significant

Growth parameters of Terminalia chebula seedlings

Overall results showed significant differences at the 5% level among different treatments applied. Data in table 3 reveals about the influence of different pre-sowing treatments on growth parameters of *Terminalia chebula* seedlings. The maximum Root length (7.70cm), shoot length (11.7cm), Number of Leaves (7.30) and Collar diameter (1.76 cm) after 45 days of sowing was obtained in GA₃ of 500 ppm for 24 hours (T₉) and on contrary minimum Root length (5.80cm), Number of Leaves (4.80) and Collar diameter (0.76 cm) was noticed in control, while shoot length (9.10cm) was found in water treatment (room temperature) for 12 hours (T₁).

The Root length, Shoot length, Number of Leaves, Collar

diameter showed significantly with increasing in the number of days and the same trend was followed after 90 days of sowing with maximum Root length (11.20cm), shoot length (14.22cm), Number of Leaves (7.81) and Collar diameter (1.93 cm) was observed in GA₃ of 500 ppm for 24 hours (T₉) conversely minimum Root length (8.40cm), Number of Leaves (5.20) and Collar diameter (0.97cm) was found in control while shoot length (10.00cm) was found in water treatment (room temperature) for 12 hours (T₁).

Comparison between root length, shoot length, collar diameter, number of leaves of 45 days with 90 days were represented under figure 1-4. Whereas the results of the study showed that Growth attributes are preferably influenced by GA₃ treatment which is very well in line with the work conducted by Thounaojam and Dhaduk (2020) [36] on Buchanania lanzan seeds where highest root length (9 cm) was obtained when seeds were treated with GA₃ @900 mg/l for 24 hrs. The importance of GA₃ in root initiation and development is evident, and the needed concentration of GA₃ may have promoted cell elongation, cell division, and cell multiplication in cambium tissues (Ratan and Reddy 2004) ^[31]. Similar results were reported by Polaiah (2020) ^[29] maximum shoot length (22.42cm) in sandalwood (Santalum album L.); Vijayakumar and Selvaraju (2013)^[40] in Cassia auriculate; Rai et al., (2018) [30] in khirni (Manilkara hexandra). Maximum number of leaves was reported by Pawar et al., (2010)^[28] in jatropha at 90 days of sowing when seeds were treated with GA₃ at 300 ppm for 8 hrs. while, maximum collar diameter (5.34 mm) was reported by Patel et al., (2018)^[27] in Santalum album when seeds treated with 500 ppm GA₃ for 24 hours and also supported by Hemalatha and chandari (2021)^[12] in the Santalum album.

Treatment details	Root length (cm)		Shoot length (cm)		Collar diameter (cm)		No. of	leaves	S: R Ratio (gm)	Dry weight of shoot (gm)	Dry weight of root (gm)
	45D	90D	45D	90D	45D	90D	45D	90D			
T 1	6.80	9.43	9.10	10.00	1.48	1.76	5.00	6.01	1.26	1.01	0.78
T ₂	6.20	9.46	9.22	10.99	1.58	1.83	4.80	6.40	1.65	1.19	0.82
T ₃	6.81	10.40	9.40	11.20	1.06	1.27	5.10	6.91	2.16	1.43	1.09
T4	5.93	9.21	9.61	10.60	1.05	1.19	5.61	6.80	2.80	1.52	0.94
T ₅	5.91	9.01	10.4	10.60	0.81	0.99	6.22	6.81	2.20	1.30	0.85
T6	7.50	11.00	11.5	12.77	1.24	1.32	6.20	7.50	2.06	1.29	0.92
T7	6.51	9.73	11.2	12.66	0.70	1.16	5.31	6.71	1.76	1.09	0.75
T8	6.82	9.83	11.5	13.22	1.21	1.43	5.83	7.32	2.51	1.75	1.30
T9	7.70	11.20	11.7	14.22	1.76	1.93	7.30	7.81	2.13	1.02	1.22
T10	5.80	8.40	9.32	11.44	0.76	0.97	4.51	5.20	1.30	0.98	0.68
SEm±	0.229	0.404	0.592	0.368	0.167	0.495	0.286	0.446	0.675	0.155	0.091
CD@5%	0.679	1.200	1.760	1.094	0.192	0.572	0.849	1.325	N/S	0.459	0.270

Table 3: Effect of different pre-sowing treatments on Growth parameters of Terminalia chebula seedlings

For biomass (shoot and root) data was taken at 90 days after sowing. Significantly highest dry weight of root (1.30g) was observed in seeds treated with 300pm GA₃ for 24 hours (T₈). While, minimum dry weight of root (0.68g) was observed in control (T₁₀). Similar results were also reported by Pawar *et al.*, (2010) ^[28] found that the dry weight of root in *Jatropha* seedlings was maximum (4.3 g) at 90 days of sowing when seeds were treated with GA₃ at 300 ppm for 8 hrs and also Ratan and Reddy (2004) ^[31] in custard apple (*Annona squamosa*).

Highest dry weight of Shoot (1.75g) was observed in seeds treated with 300ppm GA₃ for 24 hours (T₈). While, minimum Dry weight of shoot (0.98g) was observed in (T₁₀) control. This obtained result goes in line with those findings by Pawar

et al., (2010) ^[28] reported that the dry weight of shoot *jatropha* seedlings was maximum (14.3 g) at 90 days of sowing when seeds were treated with GA₃ at 300 ppm for 8 hrs and also Rai *et al.*, (2018) ^[30] in khirni (*Manilkara hexandra*).

Highest Shoot to Root ratio (2.80) was recorded in 50% conc. H_2SO_4 for 5 mins (T₄) treatment and it was superior over all other treatments performed conversely minimum Shoot to Root ratio (1.26) was recorded in (T₁) water treatment (room temperature) for 12 hours. These findings are in agreement with the findings of Fallah *et al.*, (2014) ^[9] reported the highest ratio of shoot to root (1.23) was influenced by conc. H_2SO_4 treatment which fall in harmony with the present experiment.



Fig 1: Correlating Root length of 45 days with 90 days



Fig 2: Correlating shoot length of 45 days with 90 days



Fig 3: Correlating collar diameter of 45 days with 90 days



Fig 4: Correlating no. of leaves of 45 days with 90 days

Conclusion

Terminalia chebula seeds have a limited germination capacity, lack of natural regeneration in the field due to the presence of a hard endocarp in the seed, a thick shell (endocarp), and poor kernel formation. Pre-sowing treatment is essential to enhance the germination percentage of such species having low germination either to break the dormancy or to break the hard seed coat. Among all the treatments applied, seeds of Terminalia chebula treated with water treatment (room temperature) for 24 hours and GA₃ of 500 ppm for 24 hours was found to give higher seed germination and better growth of Terminalia chebula seedlings respectively. Considering the practicability of the nursery raising technique for the species, the best treatment option obtained in this study was seeds soaked in water treatment (room temperature) for 48 hours and GA₃ of 500 ppm for 24 hours which could be useful for large scale plantation programs.

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