



Application of urea significantly enhanced the growth and biomass production in *Terminalia arjuna* seedlings

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Abstract

Terminalia arjuna, a deciduous tree native to the Indian subcontinent, is highly valued for its medicinal properties, particularly its cardioprotective and antioxidant effects. Its high nitrogen content makes it an exceptional natural source of this essential nutrient for plant growth. The current study aimed to assess the impact of urea fertilizer on the growth and photosynthetic parameters of *T. arjuna* seedlings. A pot experiment was conducted at the Department of Forestry and Range Management, University of Agriculture Faisalabad, using a completely randomized block design with five treatments and four replications. After 15 days of transplanting, urea fertilizer was applied to all pots at the recommended dose. Growth attributes measured included stem diameter, plant height, number of leaves, and root length, along with chlorophyll content. The results demonstrated that urea application significantly improved all measured growth attributes and chlorophyll content, aligning with previous research on various plant species. This research underscores the beneficial role of urea in promoting the growth and development of *T. arjuna* seedlings, contributing to the broader understanding of urea's impact on plant physiology across diverse species.

1. Introduction

Terminalia arjuna, usually well-known as Arjuna, is a large deciduous tree native to the Indian subcontinent. It belongs to the Combretaceae family and is primarily found along riverbanks and in dry deciduous forests. The tree reaches an impressive height of up to 30 meters, with a broad crown and smooth, grey bark that peels off in large, thin flakes. The root system of this plant extends outward in a radial pattern along the banks of streams, and it is relatively shallow. In open areas, this tree commonly grows as a short-trunked, low-branching species, with a sizable and rounded crown. Its bark, initially smooth on young trees, turns grey-brown and eventually peels off in thin sheets as the tree matures (Jain *et al.*, 2009; Dwivedi *et al.*, 2007).

Arjuna is sometimes cultivated in urban parts as a land tree for its attractive form, fragrant flowers, interesting foliage, and shade. Its good tolerance to saline and waterlogged soils also makes it a good candidate for seaside plantings and coastal land reclamation and rehabilitation services. To a lesser extent, the bark and fruit are important sources of tanning material for

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the leather industry. In terms of their dry weight, the young leaves contain approximately 11% crude protein content. They are harvested for feeding livestock and have traditional use as silkworm food in their native range (Morton *et al.*, 2002).

Arjuna is sometimes cultivated in urban parts as a land tree for its attractive form, fragrant flowers, interesting foliage, and shade. To a lesser extent, the bark and fruit are important sources of tanning material for the leather industry. In terms of their dry weight, the young leaves contain approximately 11% crude protein content. They are harvested for feeding livestock and have traditional use as silkworm food in their native range (Morton *et al.*, 2002). Many experimental studies have stated its progressive inotropic (Radhakrishnan *et al.*, 2001), anti-ischemic (Dwivedi *et al.*, 2000), antihypertensive (Takahashi *et al.*, 1997), anti-hypertrophic and antioxidant (Pawar and Bhutani 2005) properties, which have been studied widely (Dwivedi and Choopra 2014; Maulik and Katiyar 2010). All these properties have therapeutic relevance in the cardiovascular ailments of humans. Medical readings have described its effectiveness in various cardiovascular illnesses (Maulik and Talwar 2012), mostly in patients with ischemic heart illnesses (Bharani *et al.*, 2002; Dwivedi and Agarwal 1994), hypertension (Rao *et al.*, 2001) and heart letdown (Bharani *et al.* 1995). However, healthy planned randomized medical marks of an identical extract of arjuna in heart failure are still lacking.

The powdered bark of the arjuna tree has been discovered to possess a wide range of beneficial properties. These include cardioprotective effects, anti-ischemic action, antioxidant properties, the ability to regulate high cholesterol levels, fungicidal and antibacterial activity, antimicrobial properties, anti-inflammatory effects, immunomodulatory activity, and antinociceptive action. Furthermore, the bark powder has shown promise in treating obesity, hypertension, and hyperglycemia (Amalraj *et al.*, 2017). The enhanced antioxidant capacity of *T. arjuna* stem bark can be attributed to its higher concentration of phenolic compounds and flavonoids (Ambika *et al.*, 2014). The phytochemicals derived from *T. arjuna* can serve as a daily tonic for promoting a healthy cardiovascular system, as it is widely regarded as one of the most effective heart tonics available (Aneja *et al.*, 2012; Anjaneyulu *et al.*, 2002). Urea is a nitrogenous compound that plays an important role in agricultural practices and is widely used as a fertilizer. It is a white crystalline solid that is highly soluble in water, making it easily accessible for plant uptake. Urea is a compound of organic nature, characterized by the chemical formula $\text{CO}(\text{NH}_2)_2$ and is synthesized from ammonia and carbon dioxide. Its high nitrogen content, approximately 46%, makes it a valuable source of nitrogen for plants, supporting their growth and development (Bryson *et al.*, 2014). In terms of nitrogen fertilizers, urea makes up half of the entire production and is the most widely used because of its low cost, high nitrogen content and lack of potential for spontaneous combustion or explosions (Liu *et al.*, 2015; Naz and Sulaiman, 2017). Nitrogen leaching and ammonia gas emissions from conventional urea have the potential to harm the environment and push up production costs. Urea manufacture has become a hot-button issue around the world because of concerns about the amount of nitrogen lost in the process (Dong *et al.*, 2013). Farmers use nitrogenous fertilizers such as urea to compensate for crop N deficiency because of the strong positive reaction crops have to nitrogenous fertilizers (Ehsanullah *et al.*, 2012).

Terminalia species have explored their traditional uses in folk medicine, leading to investigations into their potential therapeutic applications. Studies have examined the effects of *Terminalia* extracts on conditions like diabetes, liver disorders, cardiovascular diseases, and skin ailments (Nair *et al.*, 2019). Furthermore, research has investigated the pharmacological mechanisms underlying the observed effects and has elucidated the pathways through which *Terminalia* compounds exert their biological activities (Patel *et al.*, 2012). Research has explored their role in reforestation, agroforestry, and habitat restoration due to their ability to enhance soil fertility, support biodiversity, and provide ecosystem services (Roy *et al.*, 2011; Kumar *et al.*, 2013). However, a little research work has been carried out on increasing the growth rate of *T. arjuna* as it is a very beneficial/important plant but it is relatively slow growing. The objective of this study was to examine the influence of urea fertilizer on the growth characteristics of *T. arjuna* seedlings.

2. Materials and Methods

2.1 Site selection

The present study was performed at the Forest Nursery and Experimental Area of the Department of Forestry and Range Management, UAF.

2.2 Planting Materials

The two-month-old saplings of *Terminalia arjuna* were collected from an experimental area of the Department of Forestry and Range Management, UAF. The healthy saplings were separated from the weaker ones. Only vigorous and healthy saplings were selected for the final experiment. All the selected saplings were homogeneous in health and stature. All Experiment units were arranged under a Complete Randomized Block Design (RCBD). The tree saplings were uniform in age and height. Silt soil samples were obtained from the Forestry Nursery and Experimental Area Department of Forestry and Range Management at UAF. Before being placed into pots, the soil was dried and then sieved to ensure uniform particle size. Each pot was filled with 5 kg of prepared soil media. The soil media was poured into the 45 earthen pots. The height and

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diameter of the vertical column were approximately 18 inches and 12 inches respectively. Two months seedlings were shifted in the pots from polythene tubes.

2.3 Fertilizer application

The plants were protected and irrigated for two weeks until they adjusted to the new environment. After three weeks the treatments were applied. A total of five treatments were applied with four replications. T0 (no fertilizer) Control, T1 (foliar spray) 1 gram, T2 (foliar spray) 2 grams, T3 (root application) 1 gram, and T4 (root application) 2 grams.

2.4 Growth parameters

The height of the plant was measured using a measuring tape, from the ground to the top of the plant. The root length was calculated using the line-measured algorithm, which involved counting the number of orthogonally and diagonally connected pairs of pixels. The measurement of root lengths was performed for each root diameter class. Stem diameter was measured at the collar (marked previously for all the individuals), using the digital Vernier caliper (INSIZE- 1112-150). The measurements were conducted one week into the experiment and continued to be recorded throughout its duration. The number of leaves for each individual in all treatment groups was manually recorded with great care. The first leaves were marked using a permanent marker, and this activity was repeated at regular one-week intervals.

2.5 Biomass production

At the end of the experiment, the individuals were removed from the study. All the leaves were collected separately, counted, and placed in paper bags. The leaves were then placed on a digital balance to calculate the fresh weight of leaves (Bsch AE-160, Germany). The fresh weight of each plant's leaf was calculated independently. Leaves of all individuals were placed in a drying oven (Memmert-110, Schwabach, USA) to measure the dry leaves weight samples. Samples of leaves packed in paper bags were dried at 60°C for five days. Later on, the dry weight of leaves was calculated with the help of digital balance (Bosch AE-160, Germany) Dry weight of the leaves of all the plants was documented.

The shoots were collected and carefully placed in paper bags. The stems were then placed on a digital balance (Bch AE-160, Germany) to measure their fresh weights. The fresh weight of the entire plant's shoots was recorded. After measuring the fresh weight of the stems from all individual plants, the stems were transferred to paper bags and placed in a drying oven (Memmert-110, Schwabach, USA) to measure the dry weight of the samples. The stem samples, packed in paper bags, were dried at 60°C for five days. Subsequently, the dry weight of the shoots was determined using a digital balance (Bosch AE-160, Germany). The dry weight of the stems from all plants was recorded for documentation purposes.

The roots were carefully separated and washed gently in water to remove any soil particles. Subsequently, the roots were placed on an analytical balance (Bosch AB-160, Germany) to measure their fresh weights. The fresh weight of the roots from all plants was recorded. After measuring the fresh weight of the roots all individuals were transferred to paper bags and placed in a drying oven (Memmert-110, Schwabach, USA) to measure the dry weight of the root samples. The root samples, packed in paper bags, were dried at 60°C for five days. Subsequently, the dry weight of the roots was determined using a digital balance (Bosch AE-160, Germany). The dry weight of the roots from all plants was recorded and documented.

The root-to-shoot ratio in plants was determined by dividing the dry weight of the roots by the dry weight of the above-ground portion of the plant. Chlorophyll contents were measured by the end of the experiment fully mature healthy leaves were selected to measure the chlorophyll contents. Chlorophyll Contents were measured with a plant leaf Chlorophyll meter. Model (at Leaf+ PN: 0131).

2.6 Statistical analysis

The collected data was analyzed using a two-way analysis of variance for species, and treatment. The means differences were tested by using the Tukey HSD test. All the means are presented along with their standard errors and all tests were taken significantly at $P < 0.005$.

3. Results

3.1 Plant height (cm)

Urea fertilizer supplementation had a substantial impact on plant height. The mean square from the analysis of data from the plant height of *Terminalia arjuna* indicates that urea application significantly increased the plant height ($P < 0.001$, Table 1). In all the treatments, plant height significantly increased by urea foliar spray as compared to control. Maximum plant height (60.9cm) was obtained under T2 followed by T1 (44.8cm), T4 (44.7cm) and T3 (40cm). While minimum plant height (34cm) was obtained under T0 (Control: no fertilizer). Statistical analyses show that results are significantly different under control and all other treatments. However, the best results were obtained under T2.

3.2 Stem diameter (mm)

Urea fertilizer supplementation had a substantial impact on stem diameter. The mean square from the analysis of data from the stem diameter of *Terminalia arjuna* indicates that urea application significantly increased the stem diameter ($P < 0.001$, Table 1). In all the treatments, stem diameter significantly increased by urea foliar spray as compared to control. Maximum

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stem diameter (6.36 mm) was obtained under T2 followed by T1 (6.05 mm), T4 (5.90 mm) and T3 (5.36 mm). While minimum stem diameter (5.09 mm) was obtained under T0 (Control: no fertilizer). Statistical analyses show that results are significantly different under control and all other treatments. However, the best results were obtained under T2.

3.3 Number of leaves

Urea fertilizer supplementation had a substantial impact on number of leaves. The mean square from the analysis of data from several leaves of *Terminalia arjuna* indicates that urea application significantly increased the number of leaves ($P < 0.001$, Table 1). In all the treatments, the number of leaves significantly increased by urea foliar spray as compared to the control. Maximum no of leaves (18.1) was obtained under T2 followed by T1 (13.12), T4 (12.7) and T3 (15). While minimum no of leaves (10.65) was obtained under T0 (Control: no fertilizer). Statistical analyses show that results are significantly different under control and all other treatments. However, the best results were obtained under T2.

Table 1: Growth parameters measurement

Treatment	T0	T1	T2	T3	T4	P-Values
Plant Height	34 (1.6) d	44.8 (1.6) b	60.9 (2.6) a	40 (2.7) c	44.7 (1.4) b	***
Stem Diameter	5.09 (0.18) c	6.05 (0.14) b	6.36 (0.34) a	5.36 (0.32) bc	5.90 (0.25) bc	***
No. of leaves	10.6 (0.67) d	13.1 (0.47) c	18.1 (0.23) a	15 (0.26) b	12.7 (0.54) c	***

3.4 Root length (cm)

Urea fertilizer supplementation had a substantial impact on root length. The mean square from the analysis of data from the root length of *Terminalia arjuna* indicates that urea application significantly increased the root length ($P < 0.001$). In all the treatments, root length significantly increased by urea foliar spray as compared to control. In all the treatments, root length significantly increased by urea foliar supply as compared to control. Maximum root length (5.9cm) was obtained under T2 followed by T1 (5.7cm), T4 (5.62cm) and T3 (5.25cm). While minimum root length (5.17cm) was obtained under T0 (Control: no fertilizer). Statistical analyses show that results are significantly different under control and all other treatments. However, the best results were obtained under T2.

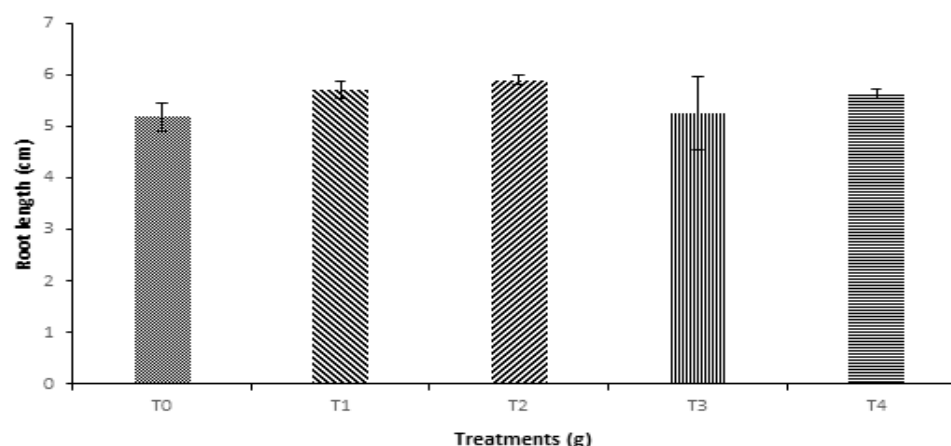


Figure: 4.1 Root length (cm) of *Terminalia arjuna* after application of different treatments

T0 = Control (no fertilizer), T1 = 1 g urea (foliar application), T2 = 2 g (foliar application), T3 = 1 g urea (root application), T4 = 2 g urea (root application).

3.5 Root shoot ratio (%)

Urea fertilizer supplementation had a substantial impact on the root shoot ratio. The mean square from the analysis of data from the root shoot of *Terminalia arjuna* indicates that urea application significantly increased the root shoot ratio ($P < 0.001$). In all the treatments, the root shoot ratio significantly increased by urea foliar spray as compared to the control. Maximum root shoot ratio (0.36 %) was obtained under T2 followed by T1 (0.35 %), T4 (0.34 %) and T3 (0.33 %). While the minimum root shoot ratio (0.31 %) was obtained under T0 (Control: no fertilizer). Statistical analyses show that results are significantly different under control and all other treatments. However, the best results were obtained under T2.

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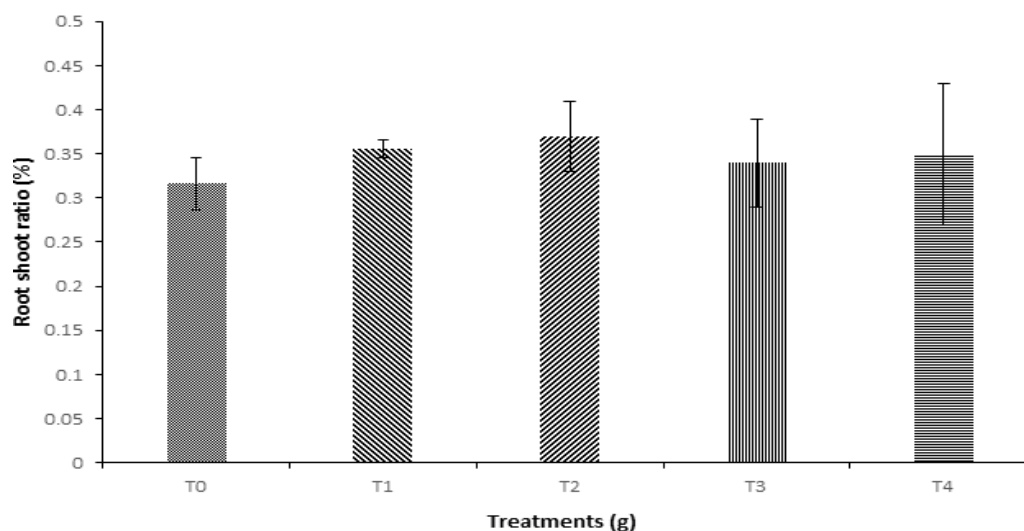


Figure: 4.2 Root shoot ratio (%) of *Terminalia arjuna* after application of different treatments

T0 = Control (no fertilizer), T1 = 1g urea (foliar application), T2 = 2g (foliar application), T3 = 1g urea (root application), T4 = 2g urea (root application)

3.6 Chlorophyll Content

Urea fertilizer supplementation had a substantial impact on chlorophyll content. The mean square from the analysis of data from the chlorophyll content of *Terminalia arjuna* indicates that urea application significantly increased the chlorophyll content ($P < 0.001$). In all the treatments, chlorophyll content significantly increased by urea foliar spray as compared to control. Maximum chlorophyll content (32) was obtained under T2 followed by T1 (20.79), T4 (27.35) and T3 (21.44). While minimum chlorophyll content (15.51) was obtained under T0 (Control: no fertilizer). Statistical analyses show that results are significantly different under control and all other treatments. However, the best results were obtained under T2.

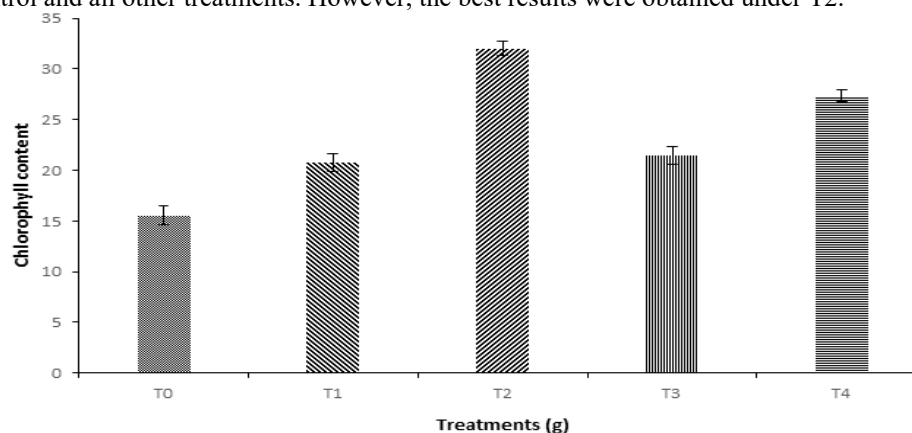


Figure: 4.3 Chlorophyll content of *Terminalia arjuna* after application of different treatments

T0 = Control (no fertilizer), T1 = 1g urea (foliar application), T2 = 2g (foliar application), T3 = 1g urea (root application), T4 = 2g urea (root application).

4. Discussion

Urea fertilizer is widely recognized for its role as a nitrogen-based fertilizer in modern agriculture. *Terminalia arjuna*, a deciduous tree native to the Indian subcontinent, is known for its high nitrogen content, which makes it an exceptional source of this vital nutrient for plants. The bark of arjuna is highly valued in traditional Ayurvedic medicine due to its cardioprotective and antioxidant properties. This study aimed to assess the impact of urea fertilizer on the growth and physiological parameters of *T. arjuna* seedlings. A pot experiment was conducted at the Department of Forestry and Range Management, UAF. The experiment was conducted using a completely randomized block design consisting of five treatments and four replications. After 15 days of transplanting, the recommended dose of urea fertilizer was applied to all the pots. The growth attributes measured included stem diameter, plant height, number of leaves, and root length. Additionally, as a physiological parameter, the chlorophyll content was also measured.

Nitrogen is an essential macronutrient required for various physiological processes, including protein synthesis and cell division (Marschner, 2012). Urea, as a highly soluble nitrogen source, readily releases nitrogen ions in the soil, making it easily accessible to plant roots. The results of this experiment are reliable with previous research on the effect of urea fertilizer on plant growth. Our results are similar to Smith et al. (2017) who reported increased growth attributes in maize plants treated with urea, while Jones et al. (2019) demonstrated the positive impact of urea on the growth of wheat seedlings. Our findings are consistent with the findings reported by Li et al. (2018) who demonstrated that urea enhanced chlorophyll content in rice seedlings and Wang et al. (2020) observed a similar increase in chlorophyll content in soybean seedlings treated with urea. These results are parallel to our study, where urea fertilizer led to a substantial increase in chlorophyll content in *Terminalia arjuna* seedlings. The positive impact of urea on chlorophyll synthesis and photosynthetic efficiency is a consistent finding across different plant species. Our conclusions exhibit similarities with the findings of Zaman et al. (2016) who examined the influence of urea on the growth of *Vachellia nilotica* seedlings and reported significant improvements in plant height, stem diameter, and leaf area. Similarly, Devkota et al. (2018) found that urea application resulted in enhanced growth attributes, including increased height and biomass accumulation, in *Dalbergia sissoo* seedlings. These findings are consistent with our results, demonstrating the growth-promoting effects of urea on *Terminalia arjuna* seedlings.

Moreover, the beneficial effects of urea fertilizer on crop growth and productivity have been extensively studied. In the context of cereal crops, such as wheat and maize, several investigations have reported positive outcomes. A study by Pandey et al. (2015) demonstrated that urea application significantly improved the growth attributes and yield of wheat plants. Similarly, Li et al. (2017) found that maize plants treated with urea exhibited increased plant height, stem diameter, and grain yield. These findings are parallel to our results, indicating the similar growth-promoting effects of urea on *Terminalia arjuna* seedlings. Our findings are similar to the results of Choudhury and Panda (2015) who examined the impact of urea on the growth and yield of tomato plants and observed significant improvements in plant height, stem girth, and fruit yield. Similarly, El-Sayed et al. (2019) reported that urea application resulted in increased growth attributes and yield in cucumber plants. These findings reinforce the understanding that urea plays a beneficial role in promoting the growth and productivity of various crops. Our results align with the findings presented by Khan et al. (2017) who explored the effects of urea on the growth of *Eucalyptus camaldulensis* seedlings and found that urea application substantially enhanced the height of the plant, biomass accumulation and stem diameter. These findings align with our results, indicating that urea has a consistent positive impact on tree growth attributes. Our findings are similar to the results of Singh et al. (2016) who studied the influence of urea on the yield and growth of chickpea plants and significant improvements were observed in terms of plant height, number of branches, and pod yield. Similarly, research by Sarker et al. (2020) demonstrated that urea application enhanced the growth attributes and yield of okra plants. These findings support our results and emphasize the beneficial effects of urea on crop growth across diverse species.

Our findings are in accordance with Das et al. (2019) who examined the impact of urea on the nodulation and growth of *Acacia mangium* seedlings and found that urea application significantly increased plant height, stem diameter, and nodule number. These results are parallel to our findings and suggest that urea fertilizer can positively influence the growth and development of nitrogen-fixing tree species. Our findings are similar to Tripathi et al. (2018) who investigated urea impact on the growth attributes of *Dalbergia sissoo* seedlings and reported significant improvements in plant height, stem diameter, and biomass accumulation. These findings are in line with our results, indicating that urea application promotes similar growth attributes in *Terminalia arjuna* seedlings. Similarly, a study by Ozel et al. (2021) examined the effects of urea on the growth of *Pinus pinea* seedlings and found that urea-treated seedlings exhibited increased shoot length, root length, and total biomass. These findings support our results and demonstrate the consistent growth-promoting effects of urea across different tree species. Our results are similar to Meena et al. (2017) who investigated the effects of urea on the growth attributes and yield of tomato plants and reported significant improvements in plant height, leaf area, and fruit yield. These findings align with our results, demonstrating that urea application promotes similar growth attributes in *Terminalia arjuna* seedlings. Similarly, research by Pal et al. (2019) demonstrated that urea treatment enhanced the growth parameters and yield of maize plants. These findings

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support our results and emphasize the beneficial effects of urea on crop growth. Our findings are similar to Kang et al. (2018) who examined the effects of urea on the growth and nitrogen fixation of soybean plants and found that urea application significantly increased plant height, nodule number, and nitrogen fixation capacity. These results are parallel to our findings, suggesting that urea fertilizer can positively influence the growth and development of nitrogen-fixing crops.

Conclusions

The study concluded that the application of urea fertilizer significantly enhances the growth and physiological parameters of *Terminalia arjuna* seedlings. Notable improvements were observed in stem diameter, plant height, number of leaves, root length, and chlorophyll content. The increased chlorophyll content suggests enhanced photosynthetic efficiency, which is crucial for overall plant health and development. Overall, the beneficial role of urea in promoting the growth and development of *T. arjuna* seedlings is evident, suggesting its potential application in sustainable forestry and agricultural practices. The positive outcomes observed underscore the importance of optimizing fertilizer use to enhance plant growth and productivity.

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