SEEFOR SOUTH-EAST EUROPEAN FORESTRY

OPEN ACCESS
ISSN 1847-6481
eISSN 1849-0891

ORIGINAL SCIENTIFIC PAPER

DOI: https://doi.org/10.15177/seefor.24-14

Investigating Light and Water Optimization for Early Seedling Establishment in *Albizia lebbeck* (L.) Benth

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Citation: Ibeh KG, Akinyele AO, 2024. Investigating Light and Water Optimization for Early Seedling Establishment in *Albizia lebbeck* (L.) Benth. *South-east Eur for* 15(2): 151-159. https://doi.org/10.15177/seefor.24-14.

Received: 28 Jan 2024; **Revised:** 30 Apr 2024; **Accepted:** 02 May 2024; **Published online:** 19 Oct 2024

ABSTRACT

Seedling growth, development, and establishment are vulnerable stages for trees, greatly influenced by two important environmental factors: light availability and soil moisture regime. This study investigated the interactive effects of light intensity and water availability on key early vegetative growth parameters of collar diameter, height, and the number of leaves in seedlings of the tropical legume tree *Albizia lebbeck*. The experiment utilized a 4×3 factorial design with four light intensity levels (L1 (75%), L2 (50%), L3 (25%), and L4 (100% as control)) and three watering frequencies (daily (W1), every three days (W2), weekly (W3)) in a nursery greenhouse. Measurements were recorded biweekly for ten weeks. The results showed that moderate light intensity of 50% coupled with adequate moisture supply by daily watering (L2W1) led to optimal seedling growth. This treatment combination resulted in the greatest mean collar diameter (3.62 mm), plant height (41.88 cm), and number of leaves (11). Growth was restricted under full light (L4) and insufficient light (L3). Limited water availability by watering weekly (W3) also suppressed growth metrics. However, there was no significant difference between daily watering (W1) and moderate watering frequency (W2). Strong intrinsic synchronization was observed between the collar diameter and plant height growth trajectories over time, reflecting coordinated lateral and vertical stem expansion regulated by developmental processes. Leaf proliferation showed partially decoupled dynamics from stem growth, indicating specialized control over leaf traits. Collar diameter, height, and the number of leaves were inherently intercorrelated, highlighting their co-dependence in generating resources and signals needed to sustain growth and development. The findings suggest that 50% light intensity and adequate moisture by watering every 1-3 days are optimal for nursery establishment of *A. lebbeck* seedlings to maximize early vegetative growth.

Keywords: *Albizia lebbeck*; light intensity; watering regime; seedling growth; growth variables; early growth; seedling morphology

INTRODUCTION

Albizia lebbeck (L.) Benth, belonging to the family Fabaceae, is a rapidly growing, nitrogen-fixing leguminous tree species native to tropical Asia that has become widely naturalized in other tropical areas (Orwa et al. 2009, Farag et al. 2013). It thrives in moderately humid and dry regions, with tropical or subtropical climates and a clear wet and dry season (Cook et al. 2005, Akinyele and Ibeh 2020). The species is highly valued for its multifunctional uses, including timber production, fodder, soil fertility enhancement, and traditional medicine derived from its bark and leaves (Mishra et al. 2010, Ibeh 2020). *Albizia lebbeck* is known for its strong ability to bind soil and prevent erosion. Its extensive but shallow root system makes it suitable for stabilizing eroded lands, such as river embankments (Orwa et al. 2009). It produces durable and attractive timber for furniture, construction, and fuel (Ibeh 2020) and bioactive compounds with anti-inflammatory, antidiabetic, antifungal, and antibacterial properties (Mishra et al. 2010).

Plant growth, development, and function are influenced by two important environmental factors: light availability and soil moisture regime (Larcher 2003). Light affects the photosynthesis, photoperiodism, and phototropism of plants (Long 2011), which in turn affects plants' stem length, leaf color, flowering, and transpiration. Light provides the energy for photosynthesis and carbohydrate production, while soil moisture sustains growth and supports key physiological processes (Taiz et al. 2018). Moisture frequency affects water availability and the stress of plants, which in turn affects their biomass, nutrient uptake, osmotic adjustment, and stomatal conductance (Chaves et al. 2003).

Seedling establishment is a vulnerable stage for trees because insufficient light or water can lead to stunted growth, developmental abnormalities, or mortality (Grossnickle 2005). Through the stomata cells, plant leaves control the movement of oxygen, carbon dioxide, and water in and out of plants. Plant closes their stomata to conserve water during water stress, thereby limiting the exchange of these components and, thus decreasing the rate of photosynthesis (Osakabe et al. 2014). On the other hand, excess moisture may delay the physiological activities of the plant. Water stress can substantially reduce productivity and survival, making moisture availability a key consideration for propagation efforts (Farooq et al. 2009). Moderate water supply is necessary for the germination and survival of plants during the first seven (7) weeks (Wilson and Witkowski 1998). It is, therefore, essential for the movement of nutrient elements, foods, and the production of carbohydrates in plants.

As interest continues to grow in planting *A. lebbeck* across the tropics for agroforestry, land restoration, and other applications, a stronger understanding of its seedling responses to variability in light and moisture availability is needed. Most of the existing studies have focused on other factors, such as temperature (Khera and Singh 2005, Lavania and Tiwari 2019), salinity (Kumar et al. 2018), and soil type (Ekaun and Ajila 2019, Olujobi et al. 2022), or on other species of the same family, such as *Leucaena leucocephala* (Tadros et al. 2012) and *Acacia sp.* (Azad et al. 2011). However, only a handful of studies (Ibeh 2020, Tomar and Shakya 2021) to date have directly investigated the effects of light intensity or water regime on early growth and developmental patterns in *A. lebbeck*. This study investigated the interactive effects of light intensity and water availability on key early vegetative growth parameters of *Albizia lebbeck*.

MATERIALS AND METHODS

Study Area

The greenhouse of the Department of Forest Production and Products, Faculty of Renewable Natural Resources, University of Ibadan, Nigeria (latitude 7°26'58"N and longitude 3°53'49"E) was used as the experimental site.

Experimental Design

The experiment was laid in 4×3 factorials in a completely randomized design (CRD) with two factors: light intensity and watering regime. Light intensity was divided into four levels: 100% (L4) (control), 75% (L1), 50% (L2), and 25% (L3) of full sunlight. The watering regime had three levels, which included once daily (W1), once every 3 days (W2), and once every 7 days (W3). We constructed wooden-framed light chambers to adjust the light intensity using green mesh nets of 1 mm thickness, which varied in number from one to three layers (Ibeh 2020). The amount of light energy that penetrated the greenhouse was measured using LI-COR 190 Quantum Sensor, which was within the electromagnetic region, ranging from 400 to 700 nm in the waveband of photosynthetic active radiation (PAR).

Polythene pots (25×13 cm) were filled with 2 kg of topsoil obtained from the nursery with a pH of 7.72. Mature and viable seeds of *Albizia lebbeck* were sourced from trees on the university premises. The seedlings in this experiment were produced generatively (seed-grown) and were transplanted at seven weeks post-planting. Forty-eight hours after transplanting, the seedlings were moved to various light chambers. We assigned 90 seedlings of similar height to each light intensity level, dividing them into three groups of 30 for different watering regimes. The seedlings were uniformly watered daily for one week to ensure proper establishment. Subsequently, they were subjected to the respective watering regimes, with each receiving 250 ml of water. Data were collected over ten weeks, culminating in an 18-week experiment from February to August 2017.

Evaluation of Growth Parameters

Growth performance was evaluated according to Ibeh (2020) by measuring three variables: plant height (cm), collar diameter (mm), and the number of leaves. We measured plant height from the soil level to the bud tip using a measuring tape, and collar diameter was measured using a digital vernier caliper. The number of leaves was counted on each seedling. These parameters were measured bi-weekly for the increase after transplant.

Linear Statistical Model

$$Y_{ijk} = \mu_i + A_j + B_k + AB_{jk} + \varepsilon_{ijk} \quad (1)$$

Where Y_{ij} is individual response obtained from the experimental research, μ_i is general mean, A_j is effects of light intensity, B_k is effects of watering regime, AB_{jk} is interactions between light intensity and watering regime, and ε_{ijk} is error term associated with the experimental design.

Experimental Layout

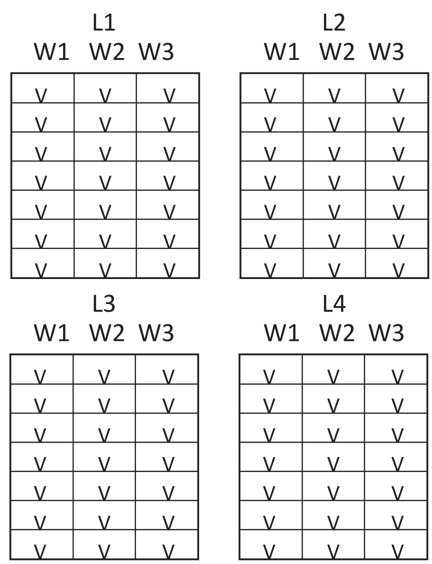

Figure 1. Nursery layout of the experiment in the light chambers.

where L1 = 75% light intensity, L2 = 50% light intensity, L3 = 25% light intensity, L4 = 100% light intensity (Control); W1 = watering once every day, W2 = watering once every 3 days, W3 = watering once every 7 days.

Statistical Analysis

We analyzed the data collected from the experiment using descriptive and inferential statistics. Descriptive statistics such as mean, standard deviation, and standard error were calculated for each growth parameter (collar diameter, plant height, and the number of leaves) under each treatment combination (light intensity and watering regime).

Inferential statistics were used to test the hypotheses and draw conclusions from the data. We performed a two-way analysis of variance (ANOVA) using the stats package (R Core Team 2023) to examine the main effects of light intensity and watering regime and their interaction on each growth parameter. The level of significance was set at 0.05. Post-hoc tests (LSD) were conducted to compare the mean differences among the levels of each factor and interaction and to identify the optimal treatment combination for each growth parameter. A correlation heatmap (corrplot package) was created to show the relationships among the variables using Pearson's correlation coefficient. We used a 3D scatter plot (scatterplot3d package) and line graph (ggplot2 package) to visualize the trend and interactions among growth variables, while a bar chart was used to display variations in time (weeks). All statistical analyses were conducted in R v 4.3.2 (R Core Team 2023).

RESULTS

The effect of varying light intensity (L1, L2, L3, and L4), watering regime (W1, W2, and W3), and their interactions on key early growth parameters of collar diameter, height, and the number of leaves were investigated in seedlings of the tropical tree *Albizia lebbeck*. Light intensities, watering regimes, and their interactions had significant effects ($p<0.05$) on all growth parameters (Figures 2a and b, Table 1). All light intensities were significantly different from each other in their effects on the growth parameters. The optimal light intensity was L2 (50% light intensity), which resulted in the highest mean values of collar diameter (3.14±0.07 mm), plant height (36.02±1.16 cm), and the number of leaves (11±0.18). The lowest mean values of these parameters were observed under L3 (25% light intensity), with values of 1.93±0.06 mm, 21.43±0.65 cm, and 8±0.17 for collar diameter, height, and the number of leaves, respectively (Figure 2a).

Watering every 3 days (W2) resulted in the greatest collar diameter (2.54±0.07 mm) and plant height (29.31±0.85 cm) and was significantly different from watering once a week (W3). The lowest growth was observed in W3, with values of 2.19±0.05 mm and 25.99±0.72 cm, and 9±0.16 for collar diameter, height, and the number of leaves, respectively. No significant difference was found between W2 and daily watering (W1). W1 and W2 had the same number of leaves and also the highest mean number of leaves (10), whereas watering once a week had the lowest value (9) (Figure 2b).

Interactive effects between light and water were significant ($P<0.05$) for all growth metrics (Table 1). Highest collar diameter (3.62±0.10 mm), height (41.88±1.85 cm), and the number of leaves (11.00±0.37) were attained under moderate light with daily watering (L2W1). L2W2 was next in performance with 3.16±0.10 mm, 35.66±2.03 cm, and 11.00±0.29, followed by L4W2 with 2.83±0.10 mm, 31.59±1.49 cm, and 10.43±0.31 while L3W1 had the least values of 1.75±0.04 mm, 17.52±0.81 cm, and 8±0.27 for collar diameter, height, and the number of leaves, respectively (Table 1).

Correlations Between Light Intensities and Watering Regimes on Early Growth of Seedlings

The correlation heatmap (Figure 3) provides insights into the relationships between the experimental factors (light intensity, and watering regime), and response variables (collar diameter, plant height, and the number of leaves).

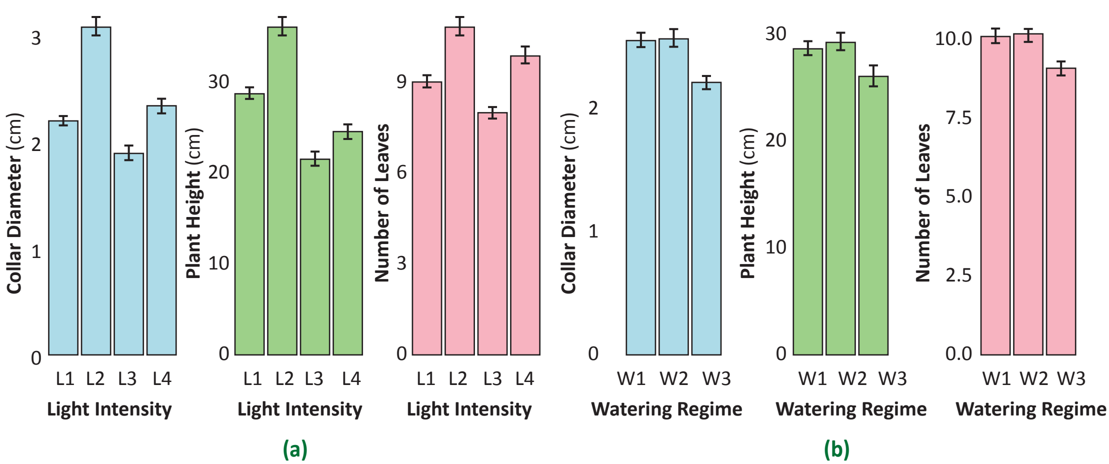

Figure 2. Effects of: **(a)** light intensities and **(b)** watering regimes on the collar diameter, height, and the number of leaves of *A. lebbeck* seedlings.

Table 1. Mean separation for the interaction effect of light intensity and watering regime on collar diameter (mm), height (cm), the number of leaves and root length (cm).

Light Intensity	Watering Regime	Collar diameter (mm)	Height (cm)	Number of leaves
L1	W1	$2.50^{a}\pm0.06$	$31.67^{a}\pm1.03$	$10^{a}\pm0.23$
L1	W2	$2.13^{b}\pm0.06$	$27.75^{b}\pm1.09$	$9^{b}\pm0.31$
L1	W3	$2.06^{c}\pm0.07$	$27.65^{c}\pm1.18$	$9^{bc}\pm0.25$
L2	W1	$3.62^{d}\pm0.10$	$41.88^{d}\pm1.85$	$11^{ad}\pm0.37$
L2	W2	$3.16^{e}\pm0.10$	$35.66^{e}\pm2.03$	$11^{ade}\pm0.29$
L2	W3	$2.62^{af}\pm0.09$	$30.51^{abcf}\pm1.66$	$10^{adef}\pm0.28$
L3	W1	$1.75^{g}\pm0.04$	$17.52^{g}\pm0.81$	$8^{bcg}\pm0.27$
L3	W2	$2.05^{bch}\pm0.14$	$22.23^{h}\pm0.90$	$8^{cgh}\pm0.30$
L3	W3	$1.97^{bcghi}\pm0.08$	$24.54^{bchi}\pm1.24$	$9^{cghi}\pm0.33$
L4	W1	$2.23^{bcghi}\pm0.08$	$21.51^{hij}\pm1.22$	$10.09^{aefj}\pm0.29$
L4	W2	$2.83^{fk}\pm0.10$	$31.59^{afk}\pm1.49$	$10.43^{adefjk}\pm0.31$
L4	W3	$2.10^{bchijl}\pm0.09$	$21.26^{gijl}\pm1.06$	$8.89^{bcgil}\pm0.26$

Values were expressed as Mean ± Standard Error. Mean values with different superscripts within the same column are significantly different (P<0.05)

As expected, there is no correlation (0) between the two experimental factors since they are independent variables in the study design. However, all three response variables are positively correlated with each other. This implies that increase in one growth parameter is also associated with increase in the other two.

The strongest positive correlation (0.85) is between collar diameter and plant height. The number of leaves is moderately positively correlated with both collar diameter (0.67) and plant height (0.62). Light intensity has weak negative correlations with collar diameter (-0.12), plant height (-0.32), and a negligible correlation with the number of leaves (-0.06). This indicates that increasing light intensity tends to restrict stem growth marginally but has a more pronounced suppression effect on leaf production. Watering regime shows a weak negative correlation with collar diameter (-0.21) and the number of leaves (-0.18), but a very weak negative relationship with plant height (-0.1). This shows that an increase in water potentially leads to a slight reduction in these parameters.

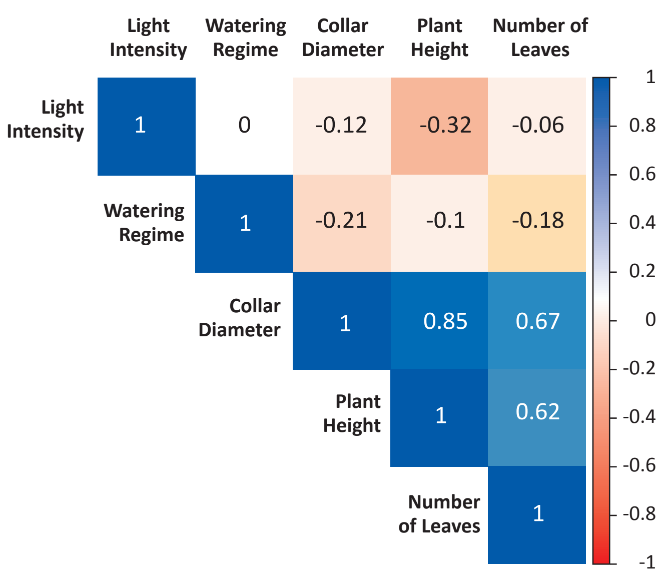

Figure 3. Correlation between light intensity, watering regime, and response variables.

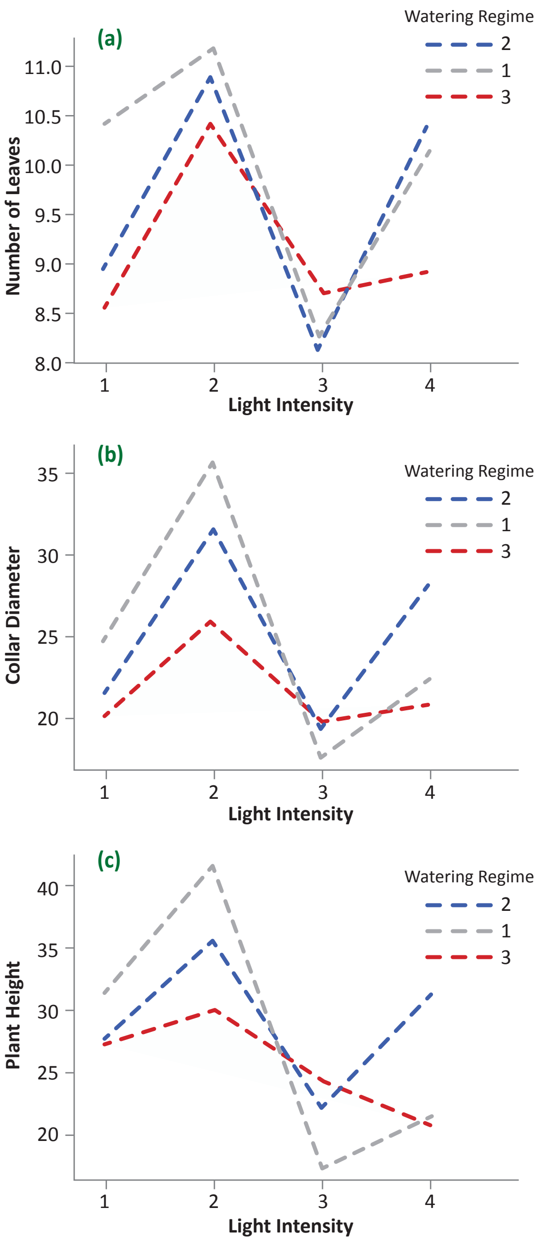

Figure 4. The combined effect of light intensities and watering regimes on the number of leaves **(a)**, collar diameter **(b)**, and plant height **(c)**.

Figure 4 (a, b, and c) visually demonstrates the interaction effects between light intensity and watering regime on the growth parameters of *A. lebbeck* seedlings. The highest values for collar diameter, plant height, and the number of leaves were consistently observed under the combination of moderate light intensity (L2) and daily watering (W1). This pattern emphasizes the importance of optimizing both light and water conditions for maximizing seedling growth. The figure also highlights the relative differences in growth parameters across the various treatment combinations, with the lowest values generally associated with low light intensity (L3) and reduced watering frequency (W3).

The 3D scatterplot illustrates the clustering and dispersion of data points, reinforcing strong positive correlations among the three growth parameters (Figure 5). The diagonal upward trend in the scatterplot indicates that higher values of collar diameter are associated with taller plant heights and a greater number of leaves. The tight clustering of data points along this trend line underscores the synchronous relationship among these variables, suggesting that they are interconnected and co-regulated in the seedling development process.

Effects of Experimental Factors on Early Growth of Lebbeck Seedlings Over Time

Figure 6 depicts the distinct patterns and trends in growth variables over the 10-week duration of the experiment. All three growth parameters showed a pattern of progressive increase over time across the varying light and moisture treatments.

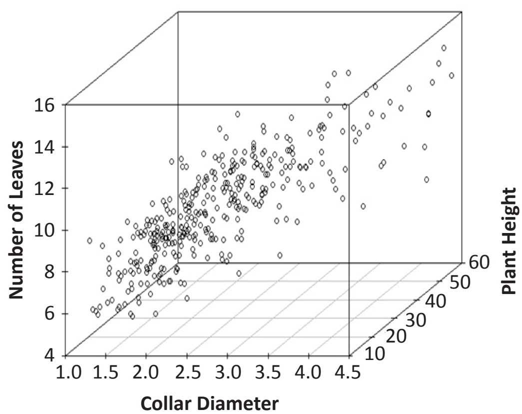

Figure 5. Synchronous relationship among the response variables.

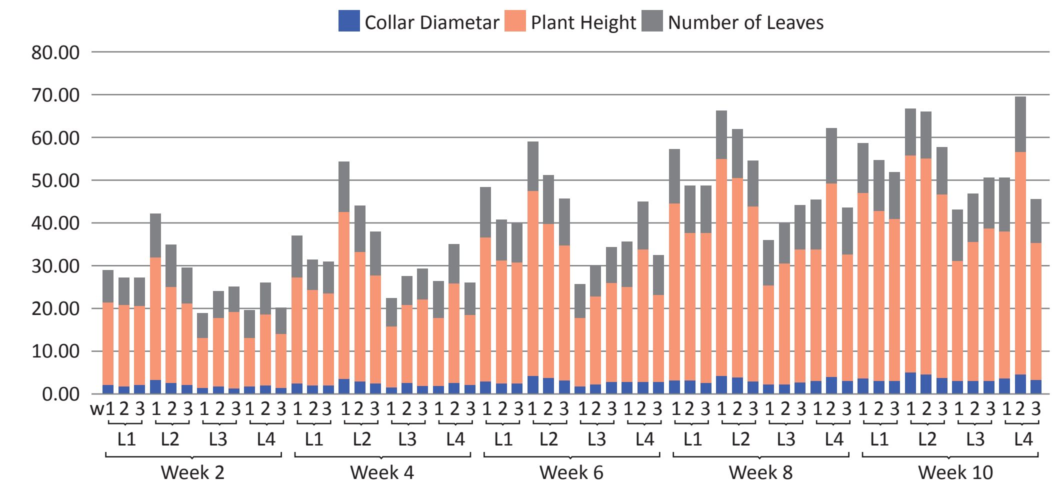

Figure 6. The trend of the experimental factors effects on growth variables throughout the experiment period.

DISCUSSION

Effects of Light Intensity on Early Growth of Seedlings

This study found that moderate light intensity led to optimal vegetative growth, resulting in the greatest collar diameter, height, and the number of leaves. This aligns with the previous studies showing that moderate irradiance balances photosynthesis and photoinhibition for maximal carbon gain and vegetative growth in tree seedlings (Zhang et al. 2022). This is attributed to the balance it provides between photosynthesis and photoinhibition, which is essential for efficient carbon assimilation and overall plant development. Moderate light intensity has been found to enhance net photosynthesis and the utilization of absorbed light, leading to optimized growth and biomass accumulation in various tree species (Sakai et al. 2011, Zhang et al. 2022). According to Albrecht and Deng (1996), plants grow towards the direction of light to expose light-capturing organs towards the light for efficient utilization of light energy.

In contrast, both low and high light intensities have been associated with limitations in photosynthetic activity and carbon gain, consequently restricting energy and materials for plant expansion (Zhang et al. 2022). Bolanle-Ojo et al. (2014) found that 100% light intensity reduced the growth and survival of *Kigelia africana* seedlings. Plants under different light chambers have greater height except for 25% light intensity (L3); this may be due to the intense shade effect on the seedlings. Low light intensity has been shown to limit photosynthetic activity, while high light intensity decreases efficiency and assimilate production, further underscoring the importance of moderate light intensity for optimal growth (Lei et al. 2005).

The study by Basyuni et al. (2020) investigated the effect of paranet shade on the growth and morphological characteristics of six species of mangrove seedlings. The results indicated that 50% paranet shade intensity supported the best growth (height and diameter) for *Ceriops tagal*, *Rhizophora mucronata*, and *Sonneratia caseolaris* seedlings. The authors attributed this to maximized light use efficiency under partial shade. High light also decreased efficiency and assimilate production, while low light limited photosynthesis. Similar optimized growth under moderate light was reported for *Dipterocarp species* seedlings, where medium light enhanced net photosynthesis and utilization of absorbed light (Sakai et al. 2014). Light intensity affects multiple physiological determinants of carbon acquisition and resource allocation that manifest through impacts on cell division and expansion, influencing seedling growth (Zhang et al. 2022, Sakai et al. 2011). This study adds further evidence that moderate light intensity synergizes these processes for optimal vegetative development in *A. lebbeck*.

Effects of Watering Regimes on Early Growth of Seedlings

Plants with sufficient moisture supply (W1 and W2) showed better growth, with a higher average number of leaves, larger collar diameter, and greater height. Adequate moisture maintains cell turgor pressure for expansion and growth, enabling nutrient mobility and carbon gain for biomass accumulation. Adequate moisture also sustains gas diffusion for continued photosynthesis and respiration to support growth. According to Majumder et al. (2010) and Taiz et al. (2018), adequate water availability helps plants retain form, structure, and maintain turgid plant cells. Our study observed that watering once in 3 days had the highest mean height and collar diameter, followed by daily watering. This agreed with the findings by Akinyele and Dada (2015) and Olajuyigbe et al. (2012) on seedlings of *Terminalia superba* and *Diospyros mespiliformis*, respectively, when subjected to different watering regimes. It was noted that moderate water supply is necessary for germination and survival at the early stage of seedling establishment, especially in the first seven weeks (Wilson and Witkowski 1998); this was confirmed by the result of our experiment. Reduced watering frequency suppressed collar expansion and height, likely by limiting cell turgor, nutrient mobility, and assimilation (Silva-Pinheiro et al. 2016).

Drought stress causes osmotic changes, tissue water deficit, and stomatal closure (Kar 2011). This reduces assimilation and growth despite adaptations like osmotic adjustment. However, overwatering can impair root function and limit growth through oxygen deficiency. It also limits gas diffusion to roots, impairing respiration and nutrient uptake (Kreuzwieser and Rennenberg 2014) and causes resources to be diverted to avoid anoxic damage rather than growth. Tadros et al. (2012), in their study on *Leucaena leucocephala*, found that the growth of seedlings in a nursery experiment improved with increased irrigation. El-Atta et al. (2012) and Sneha et al. (2012) ascertained that inadequate supply of water to plants through reduced watering frequencies resulted in reduced collar diameter, the number of leaves, height, relative growth rate, seedling dry weight and chlorophyll content of plants.

Interactive Effects of Light Intensity and Watering Regimes on Early Growth of Seedlings

The impacts of light intensity and moisture availability on key vegetative growth metrics observed in this study are linked to several physiological and morphological processes modulated by these environmental factors (Kozlowski and Pallardy 2002). Light intensity and watering regime affect multiple physiological determinants of carbon gain and resource allocation that manifest morphologically through impacts on cell division and expansion in targeted growth (Körner 2015). Stem elongation, driven by cell division in apical meristems and regulated by phytohormones such as auxin, is intricately influenced by light and moisture conditions (Kozlowski and Pallardy 1997, Landhäusser et al. 2012), which impact the hormone levels and the meristem function.

The interplay of light and water conditions may influence the cell proliferation and enlargement processes that lead to collar diameter expansion through lateral meristem activity (Kozlowski and Pallardy 2002). Increased collar diameter results from cambial cell divisions, which are also affected by moisture and light conditions. Combining optimal moderate light with adequate water availability (L2W1) synergistically maximized the growth. Intermediate growth rates were observed under combinations of L2W3 and L3W1, indicating that unsuitable levels of either factor restrict productivity even if the other resource is optimal. Conversely, full light and limited water interacted to restrict productivity most severely, thereby highlighting the importance of synchronizing light and moisture for seedling establishment.

The visual representation in Figure 4 complements the statistical analysis in Table 1, enhancing our understanding of the combined effects of light intensity and watering regime on *A. lebbeck* seedlings' growth. It highlights the sensitivity of the seedlings to these factors and helps identify the optimal combination for maximizing growth. The synchronous relationship among growth parameters, as evident in Figure 5, underscores the interconnectedness of collar diameter, plant height, and the number of leaves in the seedling development process. The strong positive correlations and tight clustering of data points along the diagonal trend line suggest that these variables are co-regulated and interdependent. Higher photosynthetic area from more leaves may contribute resources for enhanced vertical and lateral growth. The parameters increase in tandem during plant growth and development. Studies on the coordination of growth traits in tree seedlings have shown similar patterns of synchronous development and the influence of underlying regulatory mechanisms (Greenwood et al. 2001, Niinemets 2010).

The consistent growth trajectories of *A. lebbeck* seedlings over the 10-week study period illustrated in Figure 6 highlight the need to consider temporal aspects in nursery management. The steady increase in growth parameters across all treatments suggests the species' inherent capacity for sustained growth, even under suboptimal light and water conditions underscoring its resilience and adaptability. However, the varying magnitudes of growth among the treatment combinations emphasize the need to optimize light and water conditions to maximize seedling development. These findings provide valuable insights for nursery managers and reforestation efforts.

The weak negative correlations between light intensity, watering regime, and growth parameters (Figure 3) suggest complex interactions and potential optimal thresholds beyond which growth is adversely affected. The negligible correlations between watering regime and growth indicate that water availability may not directly limit vegetative growth over the study duration. In-depth studies are needed to unravel the underlying biological processes and identify confounding variables influencing these trends.

Collar diameter and height exhibited near-identical trajectories of sustained growth, revealing a strong intrinsic biological association and co-regulation between stem thickness and vertical elongation (Figure 6). The highly close parallel trends imply synchronized rates of lateral meristem expansion and vertical elongation from apical meristems. Leaf numbers also increased consistently but at a slower rate after the first four weeks. The strong correlation between the number of leaves and collar diameter/height shows that leaves provide resources to support overall plant growth. However, the partially decoupled trend suggests distinct regulatory factors for leaf production versus stem morphological processes.

These coordinated yet nuanced temporal patterns provide insights into the developmental relationships between key morphological traits underlying seedling architecture. The findings suggest common and distinct developmental regulators of collar diameter, height, and leaf number expansion over ontogeny. Explaining the endogenous and external signals modulating this coordinated vegetative phenology will advance the understanding of plant resource allocation between fundamental growth processes. This experiment provides a foundation for analyzing integrated and differential morphological responses over time under varying nursery conditions.

CONCLUSIONS AND RECOMMENDATIONS

This study provides valuable insights into the growth dynamics of *A. lebbeck* seedlings under varying environmental conditions. The findings demonstrate that both light intensity and watering regime exert significant influence on key growth parameters, including collar diameter, plant height, and the number of leaves. The optimal light intensity for seedling growth was 50% (L2), while the most favorable watering regime was found to be watering every 3 days (W2). Notably, the interaction between light intensity and watering regime played a pivotal role in determining seedling growth, with the combination of moderate light intensity (L2) and daily watering (W1) yielding the highest values for all growth parameters. These results underscore the critical importance of balancing light and water resources to achieve optimal seedling development in nursery settings.

The strong positive correlations among the growth parameters emphasized their interdependence in generating resources, materials, and signals needed to sustain coordinated plant development. We conclude that *A. lebbeck* seedlings should be grown under 50% ambient sunlight intensity and watered adequately daily during nursery establishment to optimize early vegetative growth.

Future investigations under field conditions are necessary to validate these controlled environment findings and develop site-specific light and water management practices. Follow-up studies should assess a broader range of light, moisture, temperature, and nutrient levels, coupled with growth modeling and genomic characterization.

Author Contributions

KG and AO conceived and designed the research and carried out the field measurements. KG processed the data and performed the statistical analysis, AO supervised the research and helped to draft the manuscript, KG and AO wrote the manuscript.

Funding

This research received no external funding.

Acknowledgments

I acknowledge the Department of Forest Production and Products, University of Ibadan, Nigeria for the support and for creating an enabling environment for this study.

Conflicts of Interest

The authors declare no conflict of interest.

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