

EFFECT OF MOISTURE AND LIGHT INTENSITY ON THE EARLY GROWTH OF MIRACLE BERRY (Synsepalum dulcificum (Schum. & Thonn.) Daniell): A THREATENED TROPICAL SHRUB SPECIES

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ABSTRACT

Synsepalum dulcificum is a highly valued ethnobotanical shrub, whose wild populations are threatened by over exploitation and habitat destruction. In this study, the effect of different light intensities and watering regimes on early growth of Synsepalum dulcificum were investigated for 24 weeks. In a completely randomised design experiment with factorial arrangement, uniformly growing seedlings were potted and subjected to four light intensities (25%, 50%, 75% and 100%) and four watering regimes (daily, 3-day, 5-day and 7-day intervals). The seedling total height, collar diameter and number of leaves were measured, fortnightly. The relative growth rate, absolute growth rate and net assimilation rate were also determined. Data were analysed using descriptive statistics and ANOVA at p < 0.05 level of significance. There were significant differences in the main and interaction effects of light intensity and watering regime on the growth variables. The combined effect of light intensity and watering regime revealed that 100% light intensity and watering at 7 days intervals had the highest leaf production (12.00±1.58). Seedling height increased from 6.5±0.1 cm (75% light intensity) to 6.99±0.01 cm (25% intensity). Daily watering $(7.19\pm0.1 \text{ cm})$ had the highest seedling height while 7-day watering (6.39±0.1 cm) was least. The lowest collar diameters were for 75% light intensity $(1.25\pm0.02 \text{ mm})$ and 3-day watering intervals $(1.42\pm0.02 \text{ mm})$ mm). The relative growth rate increased from 1.06x10⁻² month⁻¹ (25% light intensity) to 2.10x10⁻² month⁻¹ (75% light intensity), and 1.22x10⁻² month⁻¹ (7day watering) to 2.41x10⁻² month⁻¹ (3-day watering), respectively. Synsepalum dulcificum seedlings grew successfully at 25% light intensity and could survive for seven days without watering.

Keywords: Early growth; Underutilised species; miracle fruit; watering frequency; irradiance

INTRODUCTION

Miracle berry (*Synsepalum dulcificum* (Schum. & Thonn.) Daniell is a member of the Sapotaceae family; and also known as miracle fruit or sweet berry. Wild populations of the species are threatened by urbanisation, deforestation and expansion of arable farming

(Tchokponhoue *et al.*, 2019; Tchokponhoue *et al.*, 2021). Miracle berry is a unique fruit that changes the bitter or sour sensation in the mouth to a sweet sugar like feeling. This sweet taste lingers in the mouth for about 10 minutes to 2 hours (Joseph *et al.*, 2009; Lipatova and Campolattaro, 2016). The fleshy pulp of the fruit contains miraculin, a glycoprotein which possesses the taste modifying ability when it reacts with acid substances in the mouth (Buckmire and Francis, 1978; Tafazoli *et al.*, 2019). Hence, the red pigment in the fruit has potential use in the food and pharmaceutical industries (Lipatova and Campolattaro 2016; Priya *et al.*, 2011; Rodrigues *et al.*, 2016). Various parts of the shrub are used in traditional medicine for the treatment of different ailments such as malaria, hyperthermia, diabetes, prostate disease, tuberculosis and cough (Achigan-Dako *et al.*, 2015). In Nigeria, residual populations of *S. dulcificum* are found on abandoned land, farmlands, and home gardens. There is, therefore, an urgent need for the conservation, domestication and mass propagation of the species (Ogunsola and Ilori, 2008; Tchokponhoue *et al.*, 2019; Akinmoladun *et al.*, 2020).

Light and water are very critical for seedling growth and development, with tropical species exhibiting varying optimal and tolerance levels (Romero *et al.*, 2005; Olajuyigbe *et al.*, 2012; Onyekwelu *et al.*, 2012; Olajuyigbe and Agbo-Adediran, 2015). However, the increasing impact of climate change is expected to result in higher variations in light intensity and frequencies of drought (Poorter and Hayashida-Oliver, 2000). Hence, knowledge on tropical species' response to shade and moisture stress conditions will provide some insight on the suitable light and moisture requirements for their early developmental stages. This information would further improve the potential use of shrub species such as *Synsepalum dulcificum* in the rehabilitation or restoration of degraded and marginal lands. Furthermore, understanding the interaction effects of shade and drought on *S. dulcificum* would help explain the species' niche differentiation, and highlight its ability to survive in different microsites (Poorter and Hayashida-Oliver, 2000; Yamauchi *et al.*, 2004). Empirical information on the physiological response of the species to environmental stress would help to avoid massive failure, when the species is used in afforestation or reforestation programmes (Akinyele and Wakawa, 2017; Asinwa and Olajuyigbe, 2022).

Shade and drought may limit foliage production, leaf area index, and biomass accumulation, with excess or insufficient light and water resulting in imbalances in leaf moisture capacity and gaseous exchange between the plant and the soil (Cao, 2000; Gbadamosi, 2014; Akinyele and Wakawa, 2017). Nevertheless, response to shade and water stress are species-specific, with tropical shrub species being able to endure dry spells at certain stages of development (Poorter and Hayashida-Oliver, 2000; Poorter and Markesteijn, 2008; Tchokponhoue *et al.*, 2020). In this study, we examined the effect of different light intensities and watering regimes on the early growth characteristics and development of *Synsepalum dulcifcum* seedlings.

MATERIALS AND METHODS

Study Location

The study was conducted in the nursery of the Department of Forest Production and Products, Faculty of Renewable Natural Resources, University of Ibadan, Nigeria. The University of Ibadan is located between latitude ($7^{\circ}26'58.20''$ and $7^{\circ}26'58.08''N$) and longitude ($3^{\circ}53'48.56''$ and $3^{\circ}53'48.48''E$) at an altitude of 208 m above sea level and

approximately 160 km from the Atlantic Ocean in the southwestern region of Nigeria. The climate is West African monsoon, with high rainfall and humidity levels. The rainy season starts from April to October, while the dry season occurs from November to March. The yearly rainfall is approximately 1300 mm, while the temperature ranges from 22°C to 34°C (Olajuyigbe and Sijuola, 2022). The university campus has a high diversity of indigenous and exotic tree species including *Khaya spps, Terminalia spps, Nauclea diderrichii, Tectona grandis, Gmelina arborea* and others.

Experimental Design and Data Collection

The experiment was set up using a 4 x 4 factorial experiment in completely randomised design. Factor A (light intensity) was set at four levels (100%, 75\%, 50% and 25%), and factor B (watering regime) was set at four levels (watering daily, watering at 3-day intervals, watering at 5-day intervals and watering at 7-day intervals). To achieve different light intensity levels, one, two, or three layers of green mesh netting were used to construct light screening chambers following the methods of Olajuyigbe and Agbo-Adediran (2015). One layer of green mesh reduced light intensity by 25% allowing only 75% light to reach the seedlings; two layers of green mesh decreased light intensity by 50%, while three layers of green mesh decreased light intensity by 75%.

Ripe fruits of *Synsepalum dulcificum* were collected from a home garden in Ibadan, Nigeria. The fruits were cleaned, seeds extracted and washed, before sowing in germination beds. The germination beds were watered daily and monitored for seedling emergence. At the two-leaf development stage, the seedlings were transplanted into polythene pots (4.8 x 12 cm), containing 2 kg of topsoil and allowed to stabilize for 2 weeks. Three hundred and thirty-six uniformly growing seedlings, were then selected for the study. The seedlings were placed under the light screening chambers, while control treatment was exposed to direct sunlight. The seedlings were subjected to the different watering regimes using 100 ml of water, during each watering event. There were 16 treatment combinations with 21 replicates per treatment.

Growth variables such as number of leaves, total height, and collar diameter were measured, fortnightly for 24 weeks. Number of leaves was counted, total height was measured using a meter rule from the soil level to the tip of the apical bud, while collar diameter was measured using a digital mini vernier calliper. Five seedlings were randomly selected from each treatment combination, to determine biomass, at 2 months interval. The relative growth rate (RGR) which is the rate of seedling growth per unit time was estimated using eqn. 1. Net Assimilation Rate (NAR) which is the rate of increase in dry weight per unit leaf area was determined using eqn. 2. Absolute growth rate (AGR) was determined using eqn. 3.

$$RGR (month^{-1}) = \frac{\ln W_2 - \ln W_1}{T_2 - T_1}$$
(1)
NAR $(g \ cm^{-2} \text{month}^{-1}) = \frac{W_2 - W_1}{A_2 - A_1} X \frac{\ln A_2 - \ln A_1}{T_2 - T_1}$ (2)

AGR
$$(month^{-1}) = \frac{W_2 - W_1}{T_2 - T_1}$$
(3)

Where, A_1 = Leaf area at time 1; A_2 = Leaf area at time 2; W_1 = Leaf dry matter mass at time 1; W_2 = Leaf dry matter mass at time 2; T_1 = Time at period 1; T_2 = Time at period 2; Ln = Natural logarithm.

Data Analysis

Data on biweekly seedling growth were descriptively analysed and presented as line graphs. The main and interaction effects of light intensity and watering regime on the growth variables were determined using a two-way ANOVA at p < 0.05 level of significance. Data were transformed using square root transformation technique before subjecting to ANOVA and where significant differences exist, means were separated using the Duncan Multiple Range Test.

RESULTS

Number of Leaves

There were significant differences in the main and interaction effects of light intensity and watering regime on number of leaves. The number of leaves was highest (6.62 ± 0.16) for seedlings subjected to 100 % light intensity and least (5.48 ± 0.12) for seedlings under 75 % light intensity. Similarly, watering at 7-day intervals produced the highest number of leaves (6.78 ± 0.19) , while the least (5.69 ± 0.11) was obtained from seedlings subjected to watering at 3-day intervals (Table 1). The interaction effect of light intensity and watering regime revealed that 100% light intensity and watering at 7-day intervals had the highest number of leaves (12.00 ± 1.58) , while the least was for 75% light intensity and watering at 5-day intervals (5.03 ± 0.14) . Leaf production in seedlings watered at 7-day intervals were higher than other watering regimes at 24 weeks (Figures 1a to d).

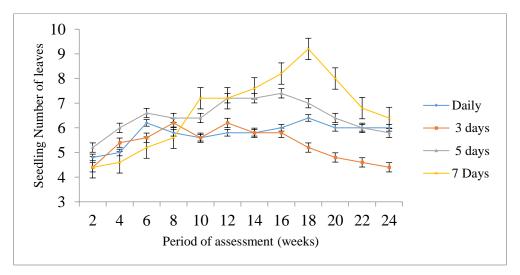
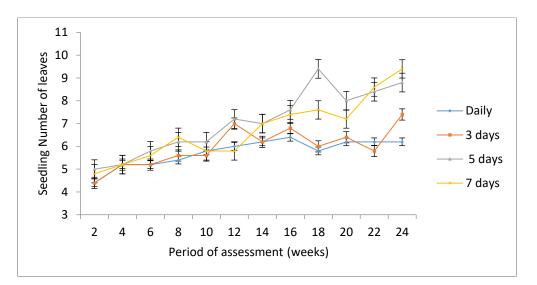


Figure 1a. Number of leaves of *Synsepalum dulcificum* seedlings exposed to 25% light intensity at varying watering regimes



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Figure 1b: Number of leaves of *Synsepalum dulcificum* seedlings exposed to 50% light intensity at varying watering regimes

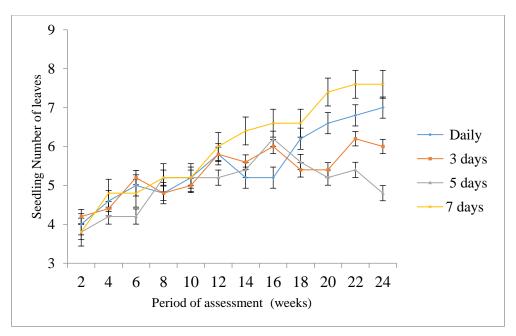


Figure 1c: Number of leaves of *Synsepalum dulcificum* seedlings exposed to 75% light intensity at varying watering regimes

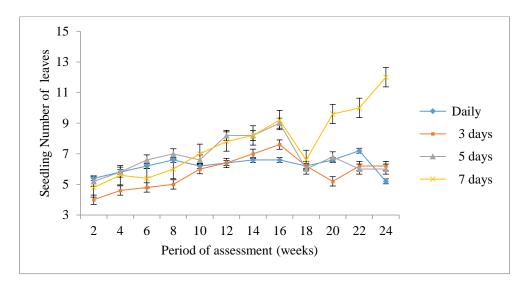


Figure 1d: Number of leaves of *Synsepalum dulcificum* seedlings exposed to 100% light intensity at varying watering regimes

Variable	Number of Leaves	Total Height (cm)	Collar Diameter (mm)
	Light	Intensity	
25%	6.07 ± 0.16^{b}	6.99±0.08 ^a	1.49±0.02 ^b
50%	6.38±0.13 ^{ab}	6.95±0.10 ^a	1.48±0.02 ^b
75%	5.48±0.12°	6.54 ± 0.09^{b}	1.25±0.02°
100%	6.62 ± 0.16^{a}	6.66 ± 0.08^{b}	1.62±0.04 ^a
	Waterin	ng Regime	
Daily	5.83±0.10 ^c	7.19±0.10 ^a	1.44±0.02
3 days	5.69±0.11°	6.61 ± 0.08^{b}	1.42±0.02
5 days	6.34±0.15 ^b	6.95 ± 0.08^{a}	1.48±0.04
7 days	6.78 ± 0.19^{a}	6.39±0.09 ^b	1.49±0.02
Interaction effect of l	light intensity and watering reg	gime	
25% x Daily	5.78±0.14 ^b	6.90±0.21 ^b	1.54±0.73
25% x 3 days	5.33 ± 0.22^{b}	6.81±0.13 ^{ab}	1.74±0.11
25% x 5 days	6.47 ± 0.31^{b}	7.41±0.11 ^{ab}	1.73±0.04
25% x 7 days	6.70 ± 0.49^{b}	6.86±0.20 ^{ab}	1.68 ± 0.15
50% x Daily	5.75 ± 0.15^{b}	9.14 ± 0.78^{a}	1.81±0.15
50% x 3 days	5.97±0.15 ^{ab}	6.45±0.14 ^{ab}	1.76±0.13
50% x 5 days	7.07 ± 0.38^{ab}	7.48±0.16 ^{ab}	1.75±0.17
50% x 7 days	6.73±0.27 ^{ab}	6.01 ± 0.16^{b}	1.71±0.21
75% x Daily	5.53±0.26 ^{ab}	7.13±0.18 ^{ab}	1.58±0.19
75% x 3 days	5.33±0.20 ^b	6.82 ± 0.18^{ab}	1.47±0.06
75% x 5 days	5.03±0.14 ^b	6.28±0.13 ^{ab}	1.60±0.13
75% x 7 days	6.00±0.30 ^{ab}	5.94 ± 0.18^{ab}	1.66±0.09
100% x Daily	6.25±0.17 ^b	6.85±0.12 ^{ab}	1.87±0.02
100% x 3 days	5.77 ± 0.30^{b}	6.37±0.20 ^b	1.67±0.18
100% x 5 days	6.78 ± 0.26^{b}	6.66±0.13 ^b	2.09±0.43
100% x 7 days	12.00 ± 1.58^{a}	6.78 ± 0.18^{ab}	1.89±0.11

Table 1: Effect of light intensity and watering regime on the growth variables of *Synsepalum dulcificum* seedlings after 24 weeks (mean ± standard error)

Means with the same letter in superscripts in each column are not significantly different ($P \le 0.05$)

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Seedling Total Height

There were significant differences in the main and interaction effects of light intensity and watering regime on seedling total height. The seedlings subjected to 25 % light intensity had the highest total height (6.99 \pm 0.08 cm), while the least (6.54 \pm 0.09 cm) was obtained for seedlings exposed to 75 % light intensity (Table 1).

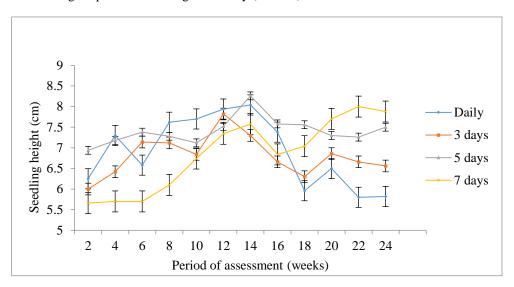


Figure 2a: Total height of *Synsepalum dulcificum* seedlings exposed to 25% light intensity at varying watering regimes

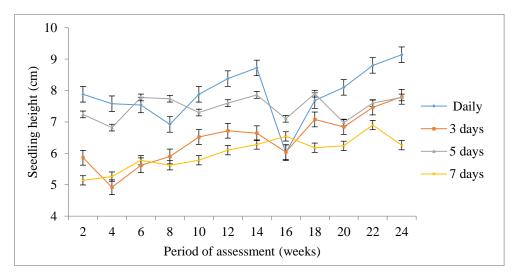


Figure 2b: Total height of *Synsepalum dulcificum* seedlings exposed to 50% light intensity at varying watering regimes

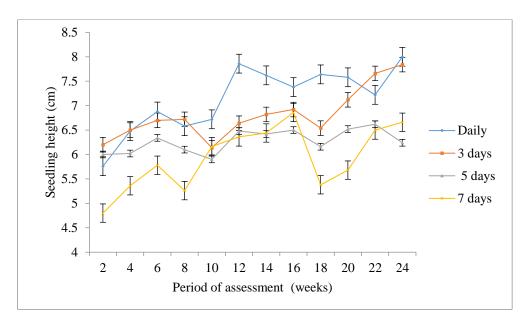


Figure 2c: Total height of *Synsepalum dulcificum* seedlings exposed to 75% light intensity at varying watering regimes

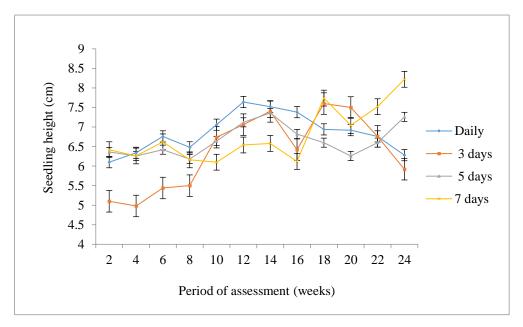


Figure 2d: Total height of *Synsepalum dulcificum* seedlings exposed to 100% light intensity at varying watering regimes

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Also, daily watering recorded the highest height $(7.19 \pm 0.10 \text{ cm})$, while the least was recorded for seedlings subjected to watering at 7-day intervals $(6.39 \pm 0.09 \text{ cm})$. The combined effect of light intensity and watering regime, resulted in seedlings subjected to 50% light intensity and daily watering having the highest total height $(9.14 \pm 0.78 \text{ cm})$, while those exposed to 50% light intensity and watering at 7-day intervals had the least $(6.01\pm0.16 \text{ cm})$. Generally, total height increment was slow over the 24-week period of the study (Figure 2a to d).

Seedling Collar Diameter

There was a significant difference in the main effect of light intensity on collar diameter. However, there was no difference in the main effect of watering regime and its interaction with light intensity. The highest collar diameter $(1.62 \pm 0.04 \text{ mm})$ was obtained for seedlings that received 100% light intensity while the least $(1.25 \pm 0.02 \text{ mm})$ was recorded for seedlings exposed to 75 % light intensity. Seedlings watered at 7-day intervals had the highest collar diameter $(1.49 \pm 0.02 \text{ mm})$, while the least $(1.42 \pm 0.04 \text{ mm})$ was observed for seedlings subjected to 3-day watering intervals (Table 1). The combined effect revealed that seedlings exposed to 100% light intensity and watering at 5-day intervals had the highest collar diameter $(2.09 \pm 0.43 \text{ mm})$, while those exposed to 75% light intensity and watering at 3-day intervals had the least $(1.47 \pm 0.06 \text{ mm})$. The increase in collar diameter followed the same trend for all treatment combinations (Figure 3a to d).

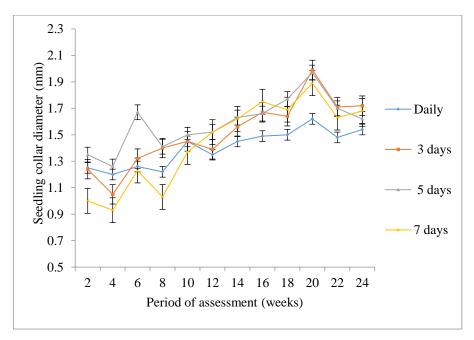


Figure 3a: Collar diameter of *Synsepalum dulcificum* seedlings subjected to 25% light intensity at varying watering regimes

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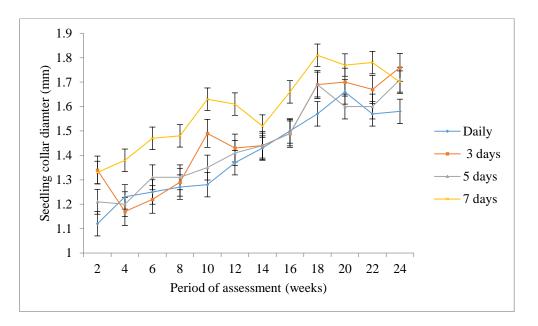


Figure 3b: Collar diameter of *Synsepalum dulcificum* seedlings subjected to 50% light intensity at varying watering regimes

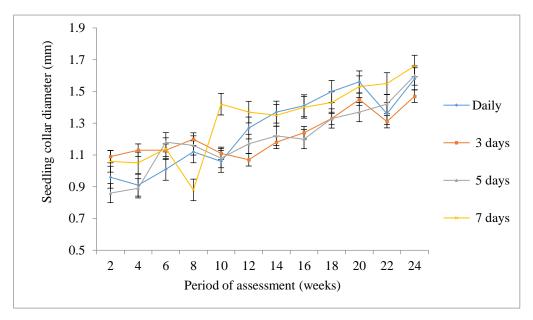


Figure 3c: Collar diameter of *Synsepalum dulcificum* seedlings subjected to 75% light intensity at varying watering regimes

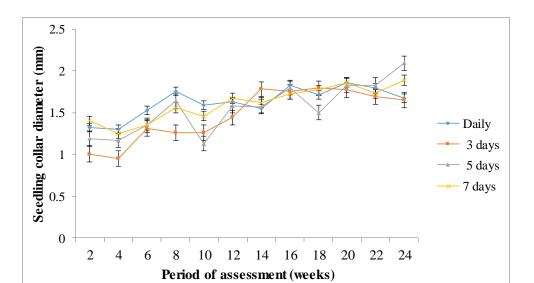


Figure 3d: Collar diameter of *Synsepalum dulcificum* seedlings subjected to 100% light intensity at varying watering regimes

Functional Growth Analysis

After 24 weeks, seedlings exposed to 75% light intensity had the highest RGR (2.10 x 10^{-2} month⁻¹), while the least RGR (1.06 x 10^{-2} month⁻¹) was obtained for those exposed to 25% light intensity (Table 2). The watering regime resulted in the highest RGR for seedlings watered at 3-day intervals (2.41 x 10^{-2} month⁻¹), while those watered at 7-day intervals had the least RGR (1.22 x 10^{-2} month⁻¹) (Table 2). For the combined effect, *S. dulcificum* seedlings raised under 75% light intensity and watered at 3-day intervals had the highest RGR (4.33 x 10^{-2} month⁻¹), while the least RGR (1.13 x 10^{-3} month⁻¹) was obtained for seedlings exposed to 75% light intensity and 7-day watering intervals.

Furthermore, seedlings subjected to 75% light intensity had the highest AGR (1.75 x $10^{-2} \text{ month}^{-1}$), while the least (1.09 x $10^{-2} \text{ month}^{-1}$) was obtained for those exposed to 25% light intensity. The seedlings subjected to 3-day watering intervals had the highest AGR (2.92 x $10^{-2} \text{ month}^{-1}$), while those subjected to 7-day watering intervals had the least (1.12 x $10^{-2} \text{ month}^{-1}$) (Table 2). The interaction between the environmental factors resulted in seedlings exposed to 75% light intensity and 3-day watering intervals recording the highest AGR (3.26 x $10^{-2} \text{ month}^{-1}$) while seedlings exposed to 100% light intensity and 5-day watering intervals recording the least (5.25 x $10^{-3} \text{ month}^{-1}$).

Net assimilation rate was highest $(3.48 \times 10^{-4} \text{ g cm}^{-2} \text{ month}^{-1})$ for seedlings exposed to 25% light intensity, while those exposed to 75% light intensity had the least $(1.70 \times 10^{-5} \text{ g cm}^{-2} \text{ month}^{-1})$. For watering regime, seedlings subjected to 3-day watering intervals had the highest NAR (3.60 X $10^{-4} \text{ g cm}^{-2} \text{ month}^{-1})$ while the least (1.28 X $10^{-5} \text{ g cm}^{-2} \text{ month}^{-1})$ was obtained for seedlings subjected to 7-day watering intervals (Table 2). The combination of the two factors resulted in seedlings exposed to 25% light intensity and 3-day watering

intervals recording the highest NAR (1.36 x 10^{-3} g cm⁻² month⁻¹), while those exposed to 100% light intensity and 5-day watering intervals having the least (2.80 x 10^{-6} g cm⁻² month⁻¹).

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50% x 5 days 1.86 x 10 ⁻² 1.66 x 10 ⁻² 4.39 x 10 ⁻⁵					
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75% x 5 days 3.24 x 10 ⁻² 2.84 x 10 ⁻² 1.99 x 10 ⁻⁵					
75% x 7 days 1.13 x 10 ⁻³ 1.75 x 10 ⁻² 3.80 x 10 ⁻⁶					
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100% x 5 days 5.97 x 10 ⁻³ 5.25 x 10 ⁻³ 2.80 x 10 ⁻⁶					
100% x 7 days 1.86 x 10 ⁻² 1.57 x 10 ⁻² 1.74 x 10 ⁻⁵					

Table 2: Relative growth rate (RGR), absolute growth rate (AGR) and net assimilation rate (NAR) of *Synsepalum dulcificum* seedlings subjected to different light intensities and watering regimes for 24 weeks

DISCUSSION

Available light and moisture are critical to the survival, growth, and development of plant species in the tropics and influence the accumulation and distribution of photosynthates. Consequently, photosynthesis and other physiological processes such as cell expansion, foliage enlargement, chlorophyll generation, and stomatal activity require light and water to be effective (Sack and Grubb, 2002; Akinyele and Wakawa, 2017). However, the light and water requirements vary with seedling age and are assumed to be low at germination and increase with seedling development.

In this study, *S. dulcificum* seedlings survived and performed well under high and low light intensities. For instance, seedlings exposed to 100% light intensity had more leaf foliage

and slightly higher collar diameters, when compared to those under shaded conditions. This agrees with Olajuyigbe and Agbo-Adediran (2015) and Akinyele and Wakawa (2017), who reported significant increases in number of leaves and collar diameters of *Entandrophragma angolense* and *Tetrapleura tetraptera* seedlings, when exposed to 100% light intensity. Tropical plants have the capacity to maximize photosynthetic activity resulting in leaf production and growth at maximum light availability.

The effects of shade on young seedlings depend on the type of species and environment, and their establishment could be encouraged or hindered depending on tolerance levels during the juvenile growth periods (Romero *et al.*, 2005, Onyekwelu *et al.*, 2012). *Synsepalum dulcificum* seedlings demonstrated a wide shade tolerance threshold because low light intensities (25% and 50%) did not inhibit leaf production, collar diameter and height development. In fact, the significant increase in height under shade (25% light intensity) could be due to reduced temperature and oxidative stress, which enhances growth (Poorter and Hayashida-Oliver, 2000; Olajuyigbe and Agbo-Adediran, 2015). Furthermore, RGR, AGR and NAR were not affected by changes in light intensity. This suggest that biomass allocation was independent of light intensity and that *S. dulcificum* seedlings were able to utilise available light for optimum growth at the early stage of development (Tchokponhoue *et al.*, 2018).

Water is required by plants for a variety of physiological activities including cell division, cell elongation and stem expansion. The investigation of plant morphological and physiological growth under low-water conditions helps with the identification of candidate species suitable for planting in water-stressed ecosystems (Cao, 2000; Olajuyigbe *et al.*, 2012; Akinyele and Wakawa, 2017). The availability of water for seedling growth is essential for successful tropical seedling production because excessive evapotranspiration may cause high mortality.

Result of this study revealed that *S. dulcificum* exhibited the ability to cope with reduced water availability (a 7-day dry spell), as this did not inhibit the survival and growth of the seedlings. In fact, leaf production slightly increased with mild water stress and there was no negative effect on the collar diameter growth. Some authors have attributed this ability to tolerate moisture stress to the slow growth of many tropical species (Olajuyigbe *et al.*, 2012; Elhadi *et al.*, 2013). On the other hand, watering frequency had a positive effect on seedling height. Nevertheless, this did not increase RGR, AGR and NAR, probably due to the slow growth and the short period of the study.

The interaction of light intensity and watering regime on *S. dulcificum* seedlings revealed the importance of these environmental factors during the early growth and development. The number of leaves and collar diameter increased at high light intensity (100%) and water stress (5- and 7-day intervals). There was a physiological response by the plant to maximize the utilisation of available solar radiation for photosynthesis, when moisture supply was limiting. This agrees with the assertions of Akinyele and Wakawa (2017), Elhadi *et al.* (2013) and Gbadamosi (2014). Biomass allocation to foliage production in low light intensity and restricted moisture improves light capture for photosynthesis (Sack and Grubb, 2002; Poorter and Markesteijn, 2008). Moderate shade (50%) and daily watering increased seedling height growth, probably due to a reduction in the effect of evapotranspiration on growth of the species (Elhadi *et al.*, 2013; Carneiro *et al.*, 2015). The absolute and relative growth rates were highest for seedlings exposed to 75% light intensity and 3-day watering intervals. This further suggests that *S. dulcificum* would probably experience optimum growth under moderate light and low water supply conditions. The

ability of the species to adapt to varying light and water supply portrays its potential use in drought prone regions, and agroforestry practices.

CONCLUSION

Synsepalum dulcificum is a slow growing shrub species, which was well adapted to different light intensities and watering regimes. The study revealed that the species could survive and grow under low light and moisture availability. The variations in the performance of the species revealed its potential as a species that could grow in different ecological zones and under various environmental conditions. This implies that the loss of wild populations of the species could be countered through the introduction of the species to home gardens, farmlands, agroforestry plots and secondary or degraded forests. Nevertheless, the outcomes of this study covered a short period in the early growth stage of the species. Hence, long term research under different field conditions would be required to categorically ascertain the physiological response of the species to different environmental conditions.

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