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GROWTH RESPONSE OF *Sysepalum dulcificum* SEEDLINGS TO WATERING REGIMES AND NITROGEN-BASED FERTILIZERS

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ABSTRACT

The experiment was carried to determine the effect of selected nitrogen based fertilizer and watering regimes on the growth variables in Synsepalum dulcificum seedlings. The study was carried out at the Forest Nursery of the Federal University of Agriculture Abeokuta, Ogun State, Nigeria. Seedlings were subjected to treatments such as Urea, NaNO3 and cow dung as fertilizer source and a control while, watering daily (7/7), every other day (3/7) and weekly (1/7) were the watering frequencies employed and these were laid out in a 3x4 factorial in CRD with 5 replicates in each treatment. Collected data was subjected to Analysis of Variance (ANOVA) and significant means were separated using LSD. Obtained results showed that height (12.45 cm), collar diameter (1.42 mm), fresh weight (0.70 g) and total dry weight (0.22 g) were significantly (p<0.05) higher in seedlings raised with cow dung while daily watering enhanced (p<0.05) collar diameter (1.43 mm) and fresh weight (0.63g). Application of cow dung and daily watering showed the best method to enhance the growth rate of Synsepalum dulcificum.

Keywords: Growth rate, watering, fertilizer, *Synsepalum dulcificum*

INTRODUTION

Fertilization is one of the silvicultural techniques and most critical components of producing high-quality seedlings in the nursery before planting out in the field. Without a good application of the required nutrient by seedlings, growth will be slow paced and seedling vigour will be reduced but with the appropriate fertilization, growth rates can be promoted up to three to five times greater than normal (Jacob and Landis, 2009). Therefore, to achieve desired growth rates, nursery plants must rely on root uptake of nutrients from the growing medium. Photosynthesis as a physiological process, rapid growth and development of plants are dependent on the supply of adequate quantities of mineral nutrients in a balanced proportion (Jacob and Landis, 2009).

Water deficits affect almost every aspect of tree growth and development it therefore becomes the most essential component for plant organism (Klymchuk *et al.*, 2008; Pallardy, 2008). Water stress in plants occurs when there is a misappropriation (in quantity and quality) of water supply to the plant thereby causing an imbalance. Hence, for continuous performance of vital processes such as photosynthesis and nutrient uptake, water should be made continuously available at the appropriate time, quality and quantity Araya (2007). Physiological and metabolic activities will be retarded when there is deficit in sufficient water to the plant. Water deficit causes unwanted moisture stress in plants such as reducing leaf expansion, leaf water potential of plant resulting in cell turgor loss and stomata conductance. This leads to loss of transpiration and decrease in photosynthetic rate thereby reducing growth and in severe cases, it causes wilting of plant (McDonald, 1984; Jensen *et al.*, 1998; Araya, 2007). Also, plant moisture stress on seedling growth are mostly evident on roots and shoots elongation thereby increasing their ratio to be 3.5 times higher, dry weight and volume growth however, persistent severe moisture stress can either damage or kill the seedling, moderate moisture stress can be beneficial in the aspect of inducing seed dormancy (McDonald, 1984; Huang *et al*., 1985). In addition to the physiological stress caused by water deficit, Shaxson and Barber (2003) discussed the interaction that often occur between soil water and nutrients, which shows that soil water can influence the availability of nutrients and the availability of nutrients can influence the uptake of soil water and plant's resistance to drought.

Although Nitrogen is more abundant than other nutrients in forest ecosystem, yet the most limiting nutrients in growth of forest trees (William and Norman, 2008). Hence, there is a need to determine the type of nitrogenous fertilizer and rate of irrigation that will enhance the growth rate of this forest seedling *Synsepalum dulcificum*.

MATERIALS AND METHODS

Study Area

The study was carried out at the forest nursery unit of the Federal University of Agriculture, Abeokuta situated North-East of Abeokuta, in Odeda Local Government of Ogun State, latitude $7^0 10^1$N and $7^0 58^1$N, and longitude $3^0 20^1$E and $4^0 37^1$N. This site falls within the tropical lowland with two distinct seasons: the longest- wet season last for eight months and the shortest-dry season lasted four months. It is characterized with mean annual rainfall of 1250- 1500mm, and mean monthly temperature ranges between 25.7^0C in July and 30.2^0C in February (Ogun-Osun River Basin Development Authority).

Seeds collection and soil media

Seeds of *Synsepalum dulcificum* were collected from the mother trees at Akinyele Local Government Area of Oyo State, and were raised in germination boxes. Top soil used as planting medium was thoroughly mixed and passed through 2 mm sieve to get a uniform soil mixture for the seedlings. It was analyzed for its nutrient content at the department of Soil Science and Land Management Federal University of Agriculture, Abeokuta (Table 1).

Table 1: Soil Analysis

Soil macronutrients	Values
N	0.251 %
P	24.67 mg/kg
K	35.49 ppm
Ca	213.00 ppm
Mg	55.50 ppm
Na	2.49 ppm
Organic carbon	0.479 %
Organic matter	0.825 %
Exchangeable acidity	2.14 cmol/kg

Source: Laboratory Results 2015

Sixty (60) seedlings were transplanted at the rate of one seedling per pot to soil filled ploy pots of size 12cm by 24cm and were fertilized with three (3) sources of nitrogen based fertilizers namely; Urea (5g), Sodium nitrate $NaNO_3$ (10g), Cow dung (15g) and non-fertilized (control) and watered to field capacity i.e. the daily watering (7/7), 2days interval (3/7) and 7days interval (1/7). This was laid out in a 3x4 Factorial in Complete Randomized Design (CRD) as source of nitrogen based fertilizer made up the first factor while frequency of watering made up the second factor of the experiment. There were 15 seedlings per treatment with five replicates in each treatment.

Data collection

Morphological and physiological variables were taken during and after the experiment.

Seedling height- (SH), Leaf area - (LA), Collar diameter- (CD), Root length- (RL) Shoot/root ratio- (S/R), Shoot weight- (SW), Root weight- (RW), Total dry weight- (TDW), Total fresh weight- (TFW), Net assimilation rate- (NAR), Relative growth rate- (RGR), Absolute growth rate- (AGR), Chlorophyll content, Relative turgidity and Relative water content.

Morphological variables were measured on seedlings forth nightly for 12 weeks while, physiological variables were measured and derived after 12 weeks as a process of destructive analysis. Seedling height was measured from the soil level to the terminal bud of the plant, collar diameter was measured at 0.5cm above the soil level with Veneer Caliper, and leaves were counted and recorded.

Leaf area was measured both at the width and length of leaf and was calculated using Ugese *et al.* (2008c)

$$LA = (4.41 + 1.14)\ L * B……1$$

Measurement of Root to shoot ratio

Plants were removed from soil after 12 weeks and washed off any loose soil. Removed plant was blotted to remove any free surface moisture and roots were separated from the shoots by cutting at soil line. Root and shoot for each plant was separately oven dried at 70^0C for 24 hours, weighed and recorded. The root to shoot ratio was calculated

$$\text{Ratio}=\frac{dry\ weight\ of\ root}{dry\ weight\ of\ shoot} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2$$

Relative water content (RWC) was determined by obtaining the fresh weight of the plants, after which the plants were immersed in water for 24 hours, the plants were removed, surface water was blotted-off and turgid weight was recorded. The same plants were oven dried at 70^0C to a constant weight for 48 hours until constant weight was obtained and recorded. It was calculated from the following equation (Turner, 1981):

$$\text{Relative water content} = (F_{wt} - D_{wt}/T_{wt} - D_{wt})x\ 100 \ldots 3$$

$$\text{Relative Growth rate} = \frac{\log_e W_2 - \log_e W_1}{T_1 - T_2} \ldots .4$$

$$\text{Absolute growth rate} = \frac{W_2 - W_1}{T_2 - T_1} \ldots\ldots\ldots\ldots 5$$

$$\text{Net assimilation rate}=\frac{(W_2 - W_1)(\log_e A_2 - \log_e A_1)}{(A_1 - A_2)(T_2 - T_1)} \ldots\ldots\ldots\ldots 6$$

$$\text{Leaf Area Ratio}=\frac{(LA_1 - LA_2)}{(W_1 - W_2)} \ldots\ldots\ldots\ldots 7$$

Where T_2 = Final time of harvesting, T_1 = Initial time of harvesting, T_2-T_1 = Time interval between initial time and final time, A_2 = Leaf area at T_2, A_1 = Leaf area at T_1, W_2 = Total dry weight of plant at T_2, W_1 = Total dry weight of plant at T_1.

Procedure for chlorophyll Analysis

The fresh tissue were collected from the field and were ground in a mortar in the presence of excess acetone until all the colour was released from the tissue. The extract and washings were then made up to a known volume. Measurement of chlorophyll was made by direct determination of the absorbance at different wavelengths using a standard spectrophotometer (read at 400-700nm) and 80% acetone extract was measured at 663 and 645 nm in 1cm cells. The concentration was calculated using the following formulae Arnon (1949).

Total chlorophyll (mg/l) = 20.2 A_{645} + 8.02 A_{663}

Data Analysis

Data collected were subjected to statistical analysis of variances on the general linear model of SAS Software (SAS institute, inc.2013). Fisher's Least Significant Difference (LSD) was used to further separate the means that were significantly different.

RESULTS

Effect of Source of Nitrogenous fertilizer and frequency of watering on morphological variables in *Synsepalum dulcificum* seedlings

Seedlings raised with cow dung had the tallest plant (12.45 cm) with largest collar diameter (1.42 mm) and were significantly different ($p<0.05$) from seedlings raised with urea with the least effect (11.07 cm and 1.11 mm respectively) (Table 2). Slight similarity was in effect as seedlings watered daily had the largest collar diameter (1.43 mm) while the least mean (1.16 mm) was observed in seedlings watered weekly (Table 3). The interaction of nitrogenous fertilizer and watering frequencies had no significant effect ($p>0.05$) on all morphological variables (Table 4).

Effect of Source of Nitrogenous fertilizers and frequency of watering on physiological variables in *Synsepalum dulcificum* seedlings

Similarly, treatment effect was significant ($p<0.05$) on variable measured as shown on Tables 5 and 6. Seedlings fertilized with cow dung produced the highest fresh weight (0.70 g), turgid weights (0.79 g), shoot dry weight (0.13 g), root dry weight (0.10 g), total dry weight (0.22 g), relative water content (85.79 %), absolute growth rate (0.002 gwk^{-1}) and relative growth rate (0.017 $gg^{-1}wk^{-1}$) while the least effect was recorded in seedlings fertilized with urea.

Result showed that daily watering had the highest effect on seedlings fresh weight (0.63 g) and dry stem weight (0.05 g) which was not significantly different ($p>0.05$)from seedlings watered every other day (0.59 g, 0.04 g). However, daily watering was significantly different ($p<0.05$) from weekly watering which least (0.44 g, 0.03 g) enhanced these variables.

The interaction of the source of nitrogenous fertilizer and frequency of watering had no significant effect on the physiological variables measured (Table 7).

Table 2: Effect of Sources of Nitrogenous fertilizer on Morphological variables in *Synsepalum dulcificum* seedlings

Source of Nitrogen	Number of leaves	Plant Height (cm)	Collar Diameter (mm)	Leaf area (cm^2/plt)
Urea	5.58^{a}	11.07^{b}	1.11^{b}	29.96^{a}
$NaNO_3$	5.83^{a}	11.73^{ab}	1.23^{ab}	27.03^{a}
Cow dung	6.00^{a}	12.45^{a}	1.42^{a}	32.43^{a}
Control	5.67^{a}	12.33^{a}	1.37^{a}	31.80^{a}

Means within a column with the same superscripts are not significantly different ($p>0.05$), LSD $p=0.05$

Table 3: Effect of frequency of watering on Morphological variables in *Synsepalum dulcificum* seedlings

Frequency of watering	Number of leaves	Plant Height (cm)	Collar Diameter (mm)	Leaf area (cm^2/plt)
Every other day	5.69^{a}	12.01^{a}	1.25^{ab}	30.81^{a}
Once a week	5.38^{a}	11.72^{a}	1.16^{b}	27.89^{a}
Daily	6.25^{a}	11.95^{a}	1.43^{a}	32.21^{a}

Means within a column with the same superscripts are not significantly different ($p>0.05$), LSD $p=0.05$

Table 4: Effect of Interaction of source of nitrogenous fertilizer and frequency of watering on Morphological variables in *Synsepalum dulcificum* seedlings

Nitrogenous fertilizer	Frequency of watering	Number of leaves	Plant Height (cm)	Collar Diameter (mm)	Leaf area (cm^2/plt)
Urea	Every other day	5.50 [a]	11.90 [b]	1.01 [c]	26.88 [d]
	Once a week	5.75 [a]	10.88 [b]	1.07 [c]	27.49 [d]
	Daily	5.50 [a]	10.43 [b]	1.25 [c]	35.51 [d]
$NaNO_2$	Every other day	5.50 [a]	12.27 [b]	1.26 [c]	28.04 [d]
	Once a week	6.00 [a]	11.60 [b]	0.99 [c]	23.30 [d]
	Daily	6.00 [a]	11.30 [b]	1.44 [c]	29.75 [d]
Cow dung	Every other day	6.00 [a]	11.85 [b]	1.42 [c]	32.82 [d]
	Once a week	5.25 [a]	12.40 [b]	1.31 [c]	31.85 [d]
	Daily	6.75 [a]	13.10 [b]	1.51 [c]	32.64 [d]
Control	Every other day	5.75 [a]	12.02 [b]	1.33 [c]	35.52 [d]
	Once a week	4.50 [a]	12.00 [b]	1.27 [c]	28.93 [d]
	Daily	6.75 [a]	12.98 [b]	1.50 [c]	30.96 [d]

Means within a column with the same superscripts are not significantly different ($p>0.05$), LSD $p=0.05$

Table 5: Effect of Sources of Nitrogenous fertilizer on Physiological variables in *Synsepalum dulcificum* seedlings

Fertilizer	**FW**	**TW**	**SHDW**	**RDW g/plant**	**LDW**	**SDW**	**TDW**	**R/S Ratio**	**RWC (%)**	**AGR (gwk^{-1})**	**RGR ($gg^{-1} wk^{-1}$)**	**NAR ($gcm^{-2}wk^{-1}$)**
Urea	0.32^{b}	0.48^{b}	0.08^{b}	0.05^{c}	0.05^{a}	0.03^{a}	0.13^{b}	0.64 ^{b}	51.93^{b}	0.000^{b}	0.007^{b}	0.000^{a}
$NaNO_2$	0.59^{a}	0.63^{ab}	0.12^{ab}	0.07^{b}	0.07^{a}	0.045^{a}	0.18^{a}	0.74 ^{b}	82.83 ^{a}	0.001^{a}	0.012^{ab}	0.025^{a}
Cow-dung	0.70^{a}	0.79^{a}	0.13^{a}	0.10^{a}	0.08^{a}	0.05^{a}	0.22^{a}	0.75 ^{b}	85.79 ^{a}	0.002^{a}	0.017^{a}	0.002^{a}
Control	0.61^{a}	0.73^{a}	0.12^{a}	0.09^{ab}	0.07^{a}	0.047^{a}	0.20^{a}	0.76 ^{b}	77.32 ^{a}	0.002^{a}	0.015^{a}	0.024^{a}

Means within a column with the same superscripts are not significantly different (*p>0.05*), LSD *p=0.05;* FW= fresh weight, TW= turgid weight, SHDW= shoot dry weight, RDW= root dry weight, LDW= leaf dry weight, SDW= stem dry weight, TDW= total dry weight, R/S Ratio= root/shoot ratio, RWC= relative water content, AGR= absolute growth rate, RGR= relative growth rate, NAR= net assimilation rate.

Table 6: Effect of Frequency of watering on Physiological variables in *Synsepalum dulcificum* seedlings

Frequency of watering	FW	TW	SHDW	RDW g/plant	LDW	SDW	TDW	R/S Ratio	RWC (%)	AGR (gwk^{-1})	RGR($gg^{-1}wk^{-1}$)	NAR ($gcm^{-2}wk^{-1}$)
Daily	0.63^{a}	0.73^{a}	0.12^{a}	0.08 ^{b}	0.07^{c}	0.05^{a}	0.21^{d}	0.67 ^{b}	78.39 ^{e}	0.0021^{f}	0.015^{g}	0.0007 ^{f}
Every other day	0.59^{a}	0.68^{a}	0.11^{a}	0.07 ^{b}	0.07^{c}	0.04^{ab}	0.19^{d}	0.73 ^{b}	78.47 ^{e}	0.0017^{f}	0.013^{g}	0.0376 ^{f}
Once a week	0.44^{b}	0.56^{a}	0.09^{a}	0.06 ^{b}	0.06^{c}	0.03^{b}	0.17^{d}	0.77 ^{b}	66.55 ^{e}	0.0014 ^{f}	0.011^{g}	0.0008 ^{f}

Means within a column with the same superscripts are not significantly different (*p>0.05*), LSD *p=0.05;* FW= fresh weight, TW= turgid weight, SHDW= shoot dry weight, RDW= root dry weight, LDW= leaf dry weight, SDW= stem dry weight, TDW= total dry weight, R/S Ratio= root/sh ot ratio, RWC= relative water content, AGR= absolute growth rate, RGR= relative growth rate, NAR= net assimilation rate.

Table 7: Effect of Interaction of source of nitrogen and frequency of watering on physiological variables in *Synsepalum dulcificum* seedlings

Fertilizer	Frequency of watering	FW	TW	SHDW	RDW g/plant	LDW	SDW	TDW	R/S Ratio	RWC (%)	AGR (gwk^{-1})	RGR ($gg^{-1}wk^{-1}$)	NAR ($gcm^{-2}wk^{-1}$)
Urea	Every other day	0.22^{a}	0.40^{b}	0.05^{c}	0.06^{d}	0.02^{e}	0.04^{f}	0.11^{g}	0.94^{h}	39.21^{i}	0.000^{a}	0.01^{b}	0.00^{d}
	Once a week	0.39^{a}	0.50^{b}	0.05^{c}	0.09^{d}	0.03^{e}	0.06^{f}	0.14^{g}	0.53^{h}	68.85^{i}	0.001^{a}	0.01^{b}	0.00^{d}
	Daily	0.35^{a}	0.53^{b}	0.04^{c}	0.10^{d}	0.04^{e}	0.06^{f}	0.14^{g}	0.43^{h}	47.74^{i}	0.001^{a}	0.01^{b}	0.00^{d}
$NaNO_2$	Every other day	0.72^{a}	0.68^{b}	0.08^{c}	0.11^{d}	0.04^{e}	0.07^{f}	0.19^{g}	0.78^{h}	111.33^{i}	0.002^{a}	0.02^{b}	0.07^{d}
	Once a week	0.42^{a}	0.50^{b}	0.05^{c}	0.09^{d}	0.04^{e}	0.05^{f}	0.14^{g}	0.93^{h}	61.20^{i}	0.001^{a}	0.01^{b}	0.00^{d}
	Every day	0.62^{a}	0.73^{b}	0.08^{c}	0.16^{d}	0.06^{e}	0.09^{f}	0.22^{g}	0.53^{h}	84.83^{i}	0.002^{a}	0.02^{b}	0.00^{d}
CD	Every other day	0.77^{a}	0.90^{b}	0.08^{c}	0.15^{d}	0.06^{e}	0.09^{f}	0.22^{g}	0.54^{h}	80.31^{i}	0.002^{a}	0.02^{b}	0.01^{d}
	Once a week	0.52^{a}	0.63^{b}	0.09^{c}	0.11^{d}	0.04^{e}	0.07^{f}	0.20^{g}	0.88^{h}	72.58^{i}	0.002^{a}	0.02^{b}	0.00^{d}
	Daily	0.82^{a}	0.85^{b}	0.12^{c}	0.13^{d}	0.06^{e}	0.08^{f}	0.25^{g}	0.87^{h}	95.58^{i}	0.003^{a}	0.02^{b}	0.00^{d}
Control	Every other day	0.67^{a}	0.75^{b}	0.09^{c}	0.14^{d}	0.06^{e}	0.09^{f}	0.23^{g}	0.64^{h}	83.01^{i}	0.002^{a}	0.02^{b}	0.07^{d}
	Once a week	0.45^{a}	0.63^{b}	0.08^{c}	0.11^{d}	0.04^{e}	0.07^{f}	0.19^{g}	0.75^{h}	63.55^{i}	0.002^{a}	0.01^{b}	0.00^{d}
	Daily	0.72^{a}	0.80^{b}	0.10^{c}	0.11^{d}	0.05^{e}	0.07^{f}	0.21^{g}	0.86^{h}	85.41^{i}	0.002^{a}	0.02^{b}	0.00^{d}

DISCUSSION

The sources of nitrogenous fertilizer affected growth of *Synsepalum dulcificum* seedlings as these affected some of the morphological and physiological variables. It was observed that morphological variables such as plant height and collar diameter were enhanced and can be attributed to the application of cow dung as source of fertilizer (Okunomo, 2010; Uddin *et al.*, 2012). Increase in collar diameter is important because it determines the rate of root growth and hence, its survival and growth on the field. Also, weed competition may be suppressed with increasing height. According to Jacob and Landis (2009) increase in physiological variables such as fresh weight, turgid weight, dry shoot weight, dry root weight, total dry weight, relative water content, absolute growth rate and relative growth rate in the seedlings were also achievable due to the application of cow dung. The reduced growth rate recorded for urea treated seedlings might be as a result of nitrification which occurred during uptake of nitrogen according to Mariswamy *et al.* (2011).

In addition, daily watering of the seedlings increased the collar diameter, fresh weight and dry stem weight irrespective of the source of fertilizer applied and cannot be introduced in a drought prone area (Okunomo, 2010). Dry weight is a better measure of growth and it indicates that this specie will have better growth rate with increase in levels of water in the soil. Also, frequent watering is necessary as lack of soil water will also diminish nutrient availability by reducing microbial activity, which is responsible for the liberation of nitrogen, phosphorus and sulphur from soil organic matter which limits growth of physiological and morphological variables in plants (Shaxson and Barber, 2003; Hartmann *et al.*, 2005; Vandoorne *et al.*, 2012 and Hirons and Percival 2011).

The negligible effect of the interaction of fertilizer and water showed that soil water can influence the availability of nutrients and the availability of nutrients can influence the uptake of soil water and plant's resistance to drought (Shaxson and Barber, 2003)

CONCLUSION

The application of cow dung as source of nitrogen fertilizer and daily watering improved the development and growth rates of *Synsepalum dulcificum* seedlings. The non-significance difference in the interaction of source of nitrogen fertilizer and frequency of watering could however be that nitrogen fertilization and watering regime would stimulate the growth of *Synsepalum dulcificum* seedlings independently.

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