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Effect of watering regime and mycorrhizal inoculation on the growth of Baobab (*Adansonia digitata*)

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Abstract: This study was carried out to investigate the effect of watering regime and mycorrhizal inoculation on the growth of *Adansonia digitata L.* seedlings. Seedlings were inoculated with ectomycorrhizae (M_0), endomycorrrhizae (M_1), non-inoculated (M_2) and subjected to three levels of watering; daily (W_0), every other day (W_1) and once a week at pot capacity (W_2). The experimental design used was split plot experiment where mycorrhizal inoculation was the main plot treatment while watering regime was the subplot treatment. Nine experimental treatments were arranged in a 3×3 factorial experimental design and replicated 5 times. Morphological parameters such as the leaf number, collar diameter, shoot height, shoot weight, root weight as well as physiological parameters such as dry weight, fresh weight, and relative water content were measured. Data obtained were taken fortnightly for a period of 12 weeks and subjected to one-way analysis of variance. The significant mean values were compared and separated using Duncan Multiple Range Test. The result indicated that shoot height (9.13 ± 8.39 cm) was significantly different ($p>0.05$) when watered once a week. There was no significant difference in seedling relative water content among the treatments. The study suggests that the seedling growth of *Adansonia digitata* could be enhanced by ectomycorrhizal inoculation and daily watering.

Keywords: *Mycorrhizal, inoculation, watering regime, parameter*

Introduction

Adansonia digitata is a massive deciduous tree, up to 20-30 m tall with a diameter up to 2-10 m at adult age (Rahul et al., 2015). African baobab is a very long-living tree. It normally lives for about 500 years, but it is believed that some trees are up to 5000 years old (Namratha and Sahithi, 2015). The binomial name of *Adansonia digitata* was given by Linnaeus, the generic name honouring Michel Adanson who had been to Senegal in the eighteenth century to describe baobab (Adanson, 1771; Rahul et al., 2015).

Adansonia digitata is widely spread over the African savanna through natural reproduction (seeds). The seeds generally take three to five weeks to germinate (Diop et al., 2005), while plants grown from seeds start flowering after eight to twenty-three years and the mature plant (over 60 years) can produce more than 160–250 fruits per year (UNCTAD, 2005). The flowering period of baobab is very long and can be reduced to less than five years by grafting (Sidibe & Williams, 2002).

Baobab is restricted to hot, semi-arid regions, dry woodland and stony places with low rainfall (less than 1500 mm annually) (Gebauer et al., 2002) and grows on a wide range of well-drained soils, from clays to sands, but not on deep unconsolidated sands, where it is unable to obtain sufficient moisture or anchorage (Wickens & Lowe, 2008).

An increased demand for these products can lead to overexploitation of the plant. It is therefore, important to determine the factors that could lead to the successful cultivation of this commercially important tree (UNCTAD, 2005).

In overall utility, perhaps no tree on earth surpasses baobab. Baobab is considered a multi-purpose tree species, because it has multiple applications ranging from food and fodder uses, to traditional medicine, cultural purposes, and trading (De Caluwé et al., 2009; De Caluwé et al., 2010; Kamatou et al., 2011). Several classes of compounds have been identified from various parts of baobab (fruit pulp, seed oil, leaves, roots) including terpenoids, flavonoids, sterols, vitamins, amino acids, carbohydrates and lipids (Shukla et al., 2001).

Mycorrhizae are symbiotic fungus root associations. The colonization of roots by mycorrhizal fungi can benefit the host by improving nutrient and water uptake. In exchange, the host plant provides the mycorrhizal fungi carbohydrates (carbon) from photosynthesis. A substantial portion of this carbon is ultimately transferred to the rhizosphere and is estimated to account for up to 15% of the organic matter in forest soils. Ectomycorrhizae and Endomycorrhizae (Arbuscular Mycorrhizae) are two types of symbiotic relationships that exist between fungi and the roots of higher plants. Ectomycorrhizae (EM), as the name suggests, do not penetrate deep into the cortical cells of the plant. The fungi enter the root between cortical cells and frequently create a thick mantle outside of the small feeder roots that is apparent to the naked eye (Byju's, 2023). In northern temperate deciduous forests, EM fungi account for 34.1% of all fungal species, but only for 11.9% in grasslands and shrublands (Tedersoo et al., 2014). Forest tree species with ectomycorrhizae include pine, firs, spruce, hemlock, oak, hickory, alder, and beech.

On the other hand, endomycorrhiza (AM) tends to enter the root cells further and cannot be detected without a microscope. The fungi are present in 74% of angiosperm species (Brundrett, 2009) and predominate in environments that are relatively nutrient-rich (such as arable soil) or phosphorous-limited (Tedersoo & Bahram, 2019).

Forest tree species with arbuscular mycorrhizae include cedars, cypress, junipers, redwoods, maple, ash, dogwoods, sycamore, yellow-poplar, and sweetgum. Agricultural crops used by forest nurseries as cover crops also form arbuscular mycorrhizae (Amaranthus et al., 2005; Corrêa et al., 2006; Corkidi et al., 2008). Compared to AM fungi, EM fungi scavenge more successfully and farther from the host roots (Teste et al., 2020). Additionally, EM trees predominate in temperate forests.

A better understanding of the mycorrhizal system for nursery, tree species, and out planting site is needed to determine the best cultural or artificial inoculation practices (Allen, 2003).

The importance of water in the growth, development and productivity of plants cannot be overemphasized. Water is an important factor in the growth, development and productivity of plants. Hartmann et al. (2005) reported that water stress due to drought is the most significant abiotic factor limiting plant growth and development. There is variation in the quantities of soil humidity required by plants for survival, optimum growth and development. This fact, which depends considerably on the climatic condition, is the basis for classifying plants into xerophytes, mesophytes and hydrophytes. To ensure hardy crops and high yields, measures toward storing up reserves of moisture in the ground and decreasing its loss are extremely important (for example, snow retention, tilling of autumn plow land, early harrowing in the spring to retain moisture, and planting of protective forest strips). In arid regions, artificial irrigation of the land is used. However, an excess of water can be harmful to plants. When the soil is flooded there is no longer room

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in the soil's capillaries for the air necessary for root respiration and normal life functions. Furthermore, flooded soil promotes anaerobic bacterial processes, which brings about the accumulation of substances that poison the roots (Sesay, 2009; Wuebker et al., 2001).

Despite its importance, it has been recognized that there is a lack of natural regeneration in most baobab populations which has been related to severe drought events, over-exploitation (e.g. extensive leaf pruning and fruit harvesting reducing fruit production and seed dispersal), grazing, and rapid shifts in land use patterns and deforestation (Dhillion & Gustad, 2004; Assogbadjo et al., 2008; Buchmann et al., 2010; Schumann et al., 2010; Venter & Witkowski, 2013). There is, thus, a growing awareness that cultivating baobabs will be very important in satisfying future local and international demand, thereby reducing the pressure on natural baobab populations.

Literature reviews on baobab by several authors provide substantial information on the species taxonomy, distribution, properties, utilization, agronomy, agroecology, nutritional value of their food products and commercial importance. However, there is a paucity of data on the domestication potentials of *Adansonia digitata* especially as it relates to mycorrhizal inoculation and its watering requirement.

It is therefore necessary to investigate the plant's ability to thrive under flooding and drought conditions; determine the effect of mycorrhizal inoculation on the growth of *Adansonia digitata* seedlings in order to ensure proper conservation and high biomass production of *Adansonia digitata* seedlings. The proposed study is therefore necessary to provide information on the effect of watering regime and mycorrhizae inoculation on the seedling growth of *Adansonia digitata.*

Materials and Methods

The seeds were collected from the Central Nursery of Forestry Research Institute of Nigeria, Ibadan. The nursery is located between latitude 7º23′N to 7º24′N and longitude 3º51′E to 3º52′E. Topsoil was collected from Forestry Nursery, Federal University of Agriculture, Abeokuta (FUNAAB). The experiment was carried out at the Forest Nursery, FUNAAB, Nigeria (Lat. 7º10′N and 7º58′N and longitude 3º20′E and 3º37′E).

Baobab (*Adansonia digitata*) seedlings were raised from seeds in polythene pots filled with topsoil. Seedlings were inoculated with ectomycorrhizae (M_0), endomycorrhizae (Vesicular Arbuscular Mycorrhizae, VAM) (M_2), and non-inoculated (M_1), at three levels of watering namely, daily at pot capacity (W_1), every other day at pot capacity (W_2), and once a week to pot capacity (W_2). Ectomycorrhizae used was obtained at the base of eucalyptus tree species from FUNAAB while Endomycorrhizae (Arbuscular Mycorrhizae) used was obtained from soils collected from a maize farm near FUNAAB, Alabata, Ogun. The experimental design is a split plot design where the main plot treatment was mycorrhizal inoculation while the subplot treatment was the watering regime. Seedlings were arranged in Completely Randomized Design (CRD) with five replicates and allowed to stabilize for 2 weeks after which growth assessment commenced.

Morphological growth parameters such as the leaf number, collar diameter, shoot height, shoot weight, root weight as well as physiological parameters which included dry weight, fresh weight, and relative water content were estimated for the nine treatments. Plant height was measured from the collar to the tip of the apical bud using a ruler calibrated in centimeters (cm). Seedling collar diameter was measured with the aid of a digital vernier caliper, calibrated in millimeters (mm). Leaf number was obtained by physical counting.

The data obtained were subjected to analysis of variance (ANOVA) using IBM Statistical Package for social sciences (IBM SPSS 20) and means were separated using Duncan Multiple Range Test at 5% level of significance.

Results

This study shows that there was no significant difference ($p>0.05$) in the effect of mycorrhizal inoculation on morphological growth parameters of *Adansonia digitata* seedlings (Table 1). However, seedling height (SH) and leaf number (LN) had the highest mean values as 7.29 ± 6.16 cm and 5.02 ± 4.13 respectively, when inoculated with ectomycorrhizal (M_o), while root weight (RW) had the highest mean value of 4.63 ± 2.31 g when non-inoculated (M_1). Table 2 shows that the effect of mycorrhizal inoculation on the physiological parameters of *Adansonia digitata* seedlings was significantly different ($p<0.05$). However, mycorrhizal inoculation had no significant effect on the relative water content of *Adansonia digitata* seedlings. The table also showed that the highest mean values of fresh weight (FW), dry weight (DW) and relative water content (RWC) were obtained when seedlings were inoculated with ectomycorrhizal (M_0). There was no significant difference ($p>0.05$) in the effect of watering regime on morphological growth parameters of *Adansonia digitata* seedlings (Table 3). However, the highest mean values of stem collar diameter (SCD), leaf number, shoot weight (SW) and root weight were obtained in seedlings watered daily (W_0). Table 4 shows that there was no significant difference ($p>0.05$) in the effect of watering regimes on physiological parameters. It also shows that the highest mean value of fresh weight, dry weight and relative water content were obtained when the seedlings were watered daily. Table 5 shows that the interactive effect of mycorrhizal inoculation and watering regime had significant difference ($p<0.05$) on shoot height but had no significant effect on other morphological growth parameters assessed such as stem collar diameter, leaf number, shoot weight and root weight. The highest mean value of shoot height (9.13 ± 8.39 cm) was obtained when seedlings were watered daily and inoculated with endomycorrhizae. The highest mean value of leaf number (6.40 ± 6.00) was obtained when subjected to daily watering and endomycorrhizal inoculation (M_2W_0). There was significant difference ($p<0.05$) in the interactive effect of mycorrhizal inoculation and watering regime on the fresh and dry weight of *Adansonia digitata* seedlings (Table 6). However, the effect of the interaction of mycorrhizal inoculation and watering regime on the relative water content of the seedlings was not significantly different, with the highest mean value of 75.66 ± 11.68% obtained in non-inoculated seedlings subjected to daily watering (M_1W_0). Fresh weight and dry weight of seedlings had the highest mean values of 8.14 ± 3.09 g and 2.11 ± 0.78 g respectively, when inoculated with ectomycorrhizae and watered daily.

Table 1 - Effects of mycorrhizal inoculation on morphological parameters.

Treatment	SCD (mm)	LN	SH (cm)	SW (g)	RW (g)
M_0	3.15 ± 2.43^{a}	5.02 ± 4.13^{a}	7.29 ± 6.16^{a}	2.23 ± 0.59^{a}	2.78 ± 1.48^{b}
M_1	2.52 ± 1.53^{a}	5.11 ± 5.00^{a}	6.19 ± 6.10^{a}	1.59 ± 0.75^{b}	4.63 ± 2.31^{a}
M_2	2.80 ± 2.37^{a}	5.80 ± 4.99^{a}	6.19 ± 5.27^{a}	1.87 ± 0.41^{ab}	2.97 ± 1.37^{b}

Mean values with the same superscript are not significantly different (P>0.05)

Table 2 - Effects of mycorrhizal inoculation on physiological parameters

Treatment	FW (g)	DW (g)	RWC (%)
M_0	6.80 ± 2.74^a	1.85 ± 0.46^a	71.65 ± 14.27^a
M_1	4.30 ± 1.78^b	0.93 ± 0.41^b	65.42 ± 13.61^a
M_2	4.62 ± 1.60^b	1.10 ± 0.37^b	69.34 ± 15.00^a

Mean values with the same superscript are not significantly different (P>0.05)

Table 3 - Effects of watering regime on morphological parameters.

Treatment	SCD (mm)	LN	SH (cm)	SW (g)	RW (g)
W_0	3.22 ± 2.45^a	6.09 ± 4.95^a	7.17 ± 5.77^a	2.00 ± 0.43^a	3.83 ± 2.77^a
W_1	2.62 ± 2.26^a	5.07 ± 4.51^a	6.08 ± 5.25^a	2.01 ± 0.67^a	3.81 ± 2.12^a
W_2	2.64 ± 2.60^a	4.78 ± 4.65^a	6.41 ± 6.51^a	1.68 ± 0.77^b	2.73 ± 0.96^a

Mean values with the same superscript are not significantly different (P>0.05)

Table 4 - Effects of watering regime on physiological parameters

Treatment	FW (g)	DW (g)	RWC (%)
W_0	5.79 ± 2.77^a	1.48 ± 0.54^a	69.76 ± 13.18^a
W_1	5.71 ± 2.21^a	1.33 ± 0.49^a	67.92 ± 15.12^a
W_2	4.22 ± 1.72^a	1.07 ± 0.64^a	68.73± 15.19^a

Mean values with the same superscript are not significantly different (P>0.05)

Table 5 - Interactive effect of mycorrhizal inoculation and watering regime on morphological parameters

Treatment	SCD (mm)	LN	SH (cm)	SW (g)	RW (g)
M_0W_0	3.43 ± 1.20^a	5.67 ± 4.10^a	7.03 ± 5.14^a	2.0 ± 0.56^a	2.57 ± 1.35^a
M_0W_1	2.74 ± 2.64^a	4.20 ± 4.33^a	5.69 ± 5.34^c	2.37 ± 0.80^a	3.40 ± 2.15^a
M_0W_2	3.29 ± 3.05^a	5.20 ± 4.77^a	9.13 ± 8.39^a	2.31 ± 0.38^a	2.35 ± 0.67^a
M_1W_0	3.30 ± 3.13^a	6.20 ± 5.72^a	7.80 ± 7.20^a	2.10 ± 0.26^a	5.76 ± 2.43^a
M_1W_1	2.48 ± 2.27^a	5.60 ± 5.15^a	6.73 ± 6.20^{ab}	1.90 ± 0.72^a	5.34 ± 2.13^a
M_1W_2	1.78 ± 2.46^a	3.53 ± 4.84^a	4.03 ± 5.26^c	0.76 ± 0.28^b	2.77 ± 1.27^a
M_2W_0	2.92 ± 2.67^a	6.40 ± 6.00^a	6.67 ± 6.10^b	1.89 ± 0.49^a	3.17 ± 1.92^a
M_2W_1	2.63 ± 2.41^a	5.40 ± 4.94^a	5.83 ± 5.36^c	1.77 ± 0.41^a	2.66 ± 1.36^a
M_2W_2	2.85 ± 2.60^a	5.60 ± 5.13^a	6.07 ± 5.54^{bc}	1.96 ± 0.42^a	3.07± 0.90^a

Mean values with the same superscript are not significantly different (P>0.05)

Table 6 - Interactive effect of mycorrhizal inoculation and watering regime on physiological parameters

Treatment	FW (g)	DW (g)	RWC (%)
M_0W_0	*8.14 ± 3.09^a*	*2.11 ± 0.78^a*	*70.65 ± 14.98^a*
M_0W_1	*7.13 ± 2.64^{ab}*	*1.71 ± 0.503^{ab}*	*69.30 ± 13.03^a*
M_0W_2	*5.12 ± 1.97^{bc}*	*1.72 ± 0.58^{ab}*	*75.00 ± 17.22^a*
M_1W_0	*4.17 ± 1.83^d*	*1.08 ± 0.29^d*	*75.66 ± 11.68^a*
M_1W_1	*5.49 ± 1.71^{abc}*	*1.14 ± 0.39^c*	*59.82 ± 11.36^a*
M_1W_2	*3.24 ± 1.27^c*	*0.56 ± 0.30^d*	*60.77 ± 13.61^a*
M_2W_0	*5.05 ± 1.76^{bc}*	*1.24 ± 0.41^{bc}*	*62.97 ± 12.05^a*
M_2W_1	*4.51 ± 1.70^{bc}*	*1.13 ± 0.39^c*	*74.63 ± 19.04^a*
M_2W_2	*4.30 ± 1.61^{bc}*	*0.93 ± 0.33^{cd}*	*70.42 ± 13.93^a*

Mean values with the same superscript are not significantly different (P>0.05)

Discussion

The results of this experiment showed that morphological and physiological parameters of *Adansonia digitata* are influenced by watering regime and mycorrhizal inoculation. Result showed that the highest mean leaf production (6.40 ± 6.00) was obtained in seedlings watered daily and inoculated with endomycorrhizae. Although, watering regimes did not significantly affect number of fronds. This result supports the findings of Agele et al. (2017) that mycorrhizal inoculation enhanced leaf production. The result is also in accordance with Agele et al. (2016) who reported that drought stress reduced the number of leaves in tree species. Although the effect of watering regime and mycorrhizal inoculation was not significant on seedling collar diameter, however the highest mean value of seedling collar diameter (3.43 ± 1.20 mm) was obtained in seedlings watered daily and inoculated with ectomycorrhizae. This is in accordance with Kim et al. (2008), who observed that mycorrhized plants were less stressed compared to non-mycorrhized plants. Although weekly irrigated seedlings were taller than those irrigated daily and every other day, no significant difference was observed among watering regimes. The results also showed the drought tolerance trait of the species, proven by the highest mean seedling height of 9.13 ± 8.39 cm obtained in seedlings watered once a week (W_2) and inoculated with ectomycorrhizal (M_1). This supports the findings of Awotoye et al. (1992) who reported that mild water stress and mycorrhizal inoculation enhanced growth than the non-mycorrhizal. A comparison based on the interactive effect of mycorrhizal inoculation and watering regimes showed that seedlings watered once in a week (W_2) and inoculated with ectomycorrhizal (M_0) were taller (9.13 ± 8.39 cm) than the non-inoculated plants (M_2) and those inoculated with endomycorrhizal (M_1). The effect of varying watering regimes on the fresh and dry shoot weights of *Adansonia digitata* seedlings was significantly different (P<0.05). This supports the findings of Gbadamosi (2014) that there were significant differences (P<0.05) in fresh weight of seedlings under varying watering regimes. The lowest mean value of 4.22 ± 1.72g obtained for Fresh weight when seedlings were watered once in a week is in agreement with the findings of Vandoorne et al. (2012) that water stress drastically decreased seedling fresh weight. However, this is contrast to Jama et al. (2008) that *Adansonia digitata* is an important savanna tree and will thrive well when watered once in a week. The result also showed that mycorrhizal inoculation increased the fresh weight of *Adansonia digitata* seedlings. This is in support with Dudhane et al. (2011) who compared non-mycorrhizal plants fresh weight of mycorrhizal inoculated Gmelina plants and reported increased levels after 45 and 75 days of mycorrhizal inoculation. The result of this interactive effect is also in accordance with Jama et al. (2008) that mycorrhyzal inoculated seedlings were significantly different ($p<0.05$) when watered once in a week. The result of this experiment also revealed that the lowest mean dry weight (1.07 ± 0.64 g) was obtained in seedlings that received the lowest watering rate. This is in accordance with the findings of Ky-Dembele et al. (2010), that drought water stress influences plant growth and limits production. The result is also in agreement with the findings of Vandoorne et al. (2012), who reported that water stress drastically decreased seedling dry weight. However, this contradicts the findings of Sakio (2005) that flooding reduces the total dry weight increment in some plant species. Dry shoot weights of *Adansonia digitata* seedlings was significantly different ($P<0.05$). This result showed that there were significant differences ($P<0.05$) in the interactive effect of mycorrhizal inoculation and watering regimes on dry weight of seedlings. The result also showed that mycorrhizal inoculation increased the dry weight of *Adansonia digitata* seedlings. This supports the findings of Dudhane et al. (2011) that mycorrhizal inoculation increased dry weight of Gmelina plants. Oyun et al. (2010) and Henson and Harun (2007) had attributed poor vigour of growth to limitations of stomatal (closure) function and negative plant water status (low leaf water potential and relative water content) especially when plants grow under soil water deficit conditions. The result of this experiment is in contrast with these findings as there were no significant difference in the effect of different watering regimes on the relative water content of the seedlings. In addition, a significant increase in the biomass of seedlings is consistent with the report by Ibiremo et al. (2011) that mycorrhizal inoculation increased shoot biomass of tree crops. Thus, it is assumed that mycorrhizal root colonization increased nutrient assimilation by seedlings that increased their vegetative growth. Founoune et al. (2002) and Yuan et al. (2004) demonstrated that common forest tree species responded positively to both ectomycorrhizal and endomycorrhizal fungi. Comparatively, the result revealed a greater overall plant performance from ectomycorrhizal than endomycorrhizal inoculation. This contradicts the findings of Oyun et al. (2010) that plant responses were greater from endomycorrhizal than ectomycorrhizal inoculation. This variation is in accordance with Smith and Read (2008) who reported that plants are selective in their mycorrhizal association and it may be that *Adansonia digitata* is selective in its association with ectomycorrhizal.

Conclusion

It was demonstrated that the type of mycorrhizal at varying frequencies of watering regimes significantly influenced some of the important morphological growth parameters such as collar diameter, shoot height as well as physiological parameters, that is, fresh weight and dry weight. *Adansonia digitata* seedlings performed best when inoculated with ectomycorrhizal (M_0) and when subjected to daily watering (W_0). Specifically, this research suggests that mycorrhizal root colonization increased nutrient assimilation by seedlings that increased their vegetative growth. Additionally, this research proved that mycorrhizal inoculation significantly improved plant physiological responses to stress and enhanced biomass of seedlings. Thus, the result of this research thus suggests ectomycorrhizal inoculation and daily watering for the optimum production of *Adansonia digitata* seedlings. In addition, further investigation with longer periods of drought, verified by field studies may help to reveal the drought resistance traits of this species.

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