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Effect of Mycorrhizal Inoculation and Watering Regimes on the Growth Performance of Garcinia kola (Heckel) Seedlings

Hammed, R. A*., Aduradola A.M*Atanda, T.A*, Osoba, A.E*, and Lawal, O.J*

* Department of Forestry and Wildlife Management, Federal University of Agriculture, Abeokuta, Nigeria

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Abstract- The experiment was carried out at the Forest Nursery Unit of the Federal University of Agriculture, Abeokuta, to determine the effect of mycorrhizal inoculation and watering regime on the growth performance of *Garcinia kola* seedlings (Heckel). *Garcinia kola* seedlings were inoculated with no mycorrhizal (M_0), ectomycorrhizal (M1) and endomycorrhizal (M2) and watered every day (W0), every other day (W1) and once a week (W2) for a period of 12 weeks. The Experiment was laid out in a 2 × 3 factorial in Completely Randomized Design (CRD) with five (6) replications in each treatment. Data obtained were subjected to both descriptive and factorial analysis using ANOVA, while significant means were separated using Fisher's Least Significant Difference. The result indicated that leaf number (4.56) and absolute growth rate (-1.99gwk-1) were significantly influenced ($p<0.05$) in seedlings inoculated with ectomycorrhizal. Seedlings that were watered daily (W_0) recorded the highest growth in leaf number (4.41) and plant height (12.22) with no significant difference. Seedlings watered once daily with inoculation with endomycorrhizal had the maximum growth. The study concludes that irrespective of watering regime *Garcinia kola* seedlings will perform better with or without mycorrhizal. As a water-loving plant, *Garcinia kola* seedlings will perform better when watered once daily to pot capacity in terms of leave number and plant height. It can be concluded that this plant species may be selective in its mycorrhizal association.

***Index Terms*-** Watering regime, mycorrhizal inoculation, Garcinia kola, seedlings, growth rate.

I. Introduction

A mycorrhizal is a symbiotic association between a green plant and a fungus. The plant captures the energy coming from the sun by means of utilizing its chlorophyll and supplies it to the fungus which supplies water and mineral nutrients taken from the soil to the plant. Mycorrhizal plants are often more tolerant to diseases, such as those caused by microbial soil-borne pathogens. These associations have been found to assist in plant defense both above and below ground. Mycorrhizae are known to excretes enzymes that are toxic to soil borne organisms such as nematodes (Azcon-aguilar, 2018) and defense responses are stronger in plants with mycorrhizal associations (Jung *et al.*, 2012). Additionally, Babikova *et al.*, (2003) also reported that the mycorrhizal fungus assists by preventing the plant's carbon relocation which negatively affects the fungi's growth and occurs when the plant is attacked by herbivores. The absence of mycorrhizal fungi can also slow plant growth in early succession or on degraded landscapes (Jeffries *et al.*, 2003). The introduction of alien mycorrhizal plants to nutrient-deficient ecosystems puts indigenous non-mycorrhizal plants at a competitive disadvantage (David, 2000).

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. Water stress drastically decreased fresh and dry weight, leaf number, total leaf area and stomata conductance (Vandoorne, *et al.*, 2012). Plant species respond differently to water availability, also, different plant parts adapt differently to varying water stress conditions. Seeds of many crop species are sensitive to flooding stress during germination (Sesay, 2009; Wuebker *et al.*, 2001; Sung, 1995).

Garcinia kola is one of the Non-Timber Forest Products that are of high socio-economic importance in Nigeria. It is endemic to the humid lowland rainforest vegetation of the west and central African sub regions. It is found in coastal areas and lowland plains up to 300m above sea level with an average of 2000mm–2500mm rainfall per annum and temperature ranges from 32.15ºC to 21.40ºC and with minimum relative humidity of 76.34% (Raven *et al*., 2005).

Garcinia kola is one of the tropical plants found in Africa, America and Asia. These species are commonly used for many purposes (Rai, 2003). The plant grows as a medium size tree, up to 12-14m high and produces reddish yellowish or orange coloured fruit (Okwu 2005; Adesanya *et al*., 2007). Each fruit contains 2-4 yellow nuts and a sour tasting pulp. Its nuts are commonly called bitter kola (may be because the nuts when chewed have a bitter astringent taste) or false kola (since they often serve as an alternative to true kola nuts, *Cola acuminata*). Bitter kola is also known as African wonder nut. In Nigerian languages, it is commonly called 'Namijigoro' in Hausa, 'Agbilu' in Igbo, and 'Orogbo' in Yoruba. *G. kola* has economic and cultural values across West and Central African countries where the nuts are commonly chewed and used for traditional ceremonies (Eleyinmi, 2006).

In Nigeria, low populations of *G. kola* are found in home gardens and few stands are found in the wild due to rapid deforestation and heavy exploitation in the natural forests. These factors seriously depleted the populations of the species. But demand for *G. kola* is currently very high in Nigeria and though few seeds are available in the markets, production of the species is limited due to problem of seed dormancy. The seeds need to be treated to enhance germination. During a priority setting exercise, *G. kola* was one of the useful indigenous trees prioritized by farmers in West and Central Africa. For many farmers who practice agroforestry, medicinal and fruit trees turn out to have higher priority. The challenges to development institutions are to help overcome these problems and to respond to priorities of rural communities, especially in the area of conserving highly endangered and valued species. In spite of great demand for *G. kola* seeds, its cultivation is not popular owing to the difficulty in germination (Adebisi, 2004), although *G. kola* is one of the most useful indigenous tree species prioritized by farmers in Central and West Africa (Anegbeh, 2006). The study focused om the effect of different types of mycorrhiza and watering regime on the growth performance of *G. kola* (Heckel) seedlings to determine the best treatment levels as well as the most appropriate moistures needed or suitable for the growth of *G. kola* (Heckel) seedlings.

II. Materials and Method

Experimental site

The experiment was carried out in the forest nursery of the Department of Forestry and Wildlife Management in Federal University of Agriculture, Abeokuta. This area falls within the latitude 70 N and 7058' N and longitude 3020' E and 3037' E. It has a gentle slope undulating landscape and mild slope. The site is punctuated in parts by ridges, isolated, residual hills, valley, and low lands. The soils are sand and clay with crystalline basement complex. It has an annual rainfall of 1200 mm with a peak in June and July; there is a dry season of three months. The relative humidity of the area is 82.54 % and an average monthly temperature of 35.80 C.

Experimental methods and layout

A 2 x 3 factorial in CRD was the experimental design used with 6 replicates to assess the effect of mycorrhizal inoculation and watering regimes on the growth performance of *Garcinia kola* seedlings. A source of mycorrhizal which was no mycorrhizal (M_0), ectomycorrhizal (M1) and endomycorrhizal (M2) constituted factor A while the frequency of watering namely: Watering daily 7/7 (W0), Watering every other day 2/7 (W1) and Watering once a week 1/7 (W2) were the factor B. Total of nine treatments were used for this study and were represented below as M_0W_0, M_0W_1, M_0W_2, M_1W_0, M_1W_1, M_1W_2, M_2W_0, M_2W_1, and M_2W_2. Thereafter, the inoculated replicates were prepared thus: two head pans of top soil were thoroughly mixed with one head pan of ectomycorrhizal soil and two head pans of top soil were thoroughly mixed with 2kg of endomycorrhizal soil, the mixture was used to fill the polythene pots, while those that were not inoculated, top soil was used to fill their polythene pots. Sufficient quantity of water was added to the filled polythene pots and allowed to drain before transplanting. The seedlings of *Garcinia kola* were transplanted at 2-4 leaf stage into the filled drained polythene pots and watered daily to pot capacity for one week to enhance the establishment of the plants. For each replicate of the experiment at one seedling per pot, 18 seedlings were allocated for each sources of soil used while 6 seedlings were allocated to each watering regime. At the commencement of the 2nd week of transplanting, seedlings under each treatment were subjected to varying watering regime.

Data collection

During the period of growth, measurements of both the morphological and physiological parameters were taken fortnightly for 12 weeks. Morphological parameters include; seedling heights, collar diameter, leaf number, leaf area. The physiological parameters measured at the end of the experiment were; relative water content, chlorophyll content and leaf turgidity.

Fresh weight

After twelve weeks of experimental treatments, the seedlings of each species were harvested from the pot and were separated into leaf, root, and stem. Root and shoot length was measured with a ruler, fresh weight of each component were taken with sensitive weighing balance and then the average were recorded

Dry matter

Dry weights of each component (leave, root and shoot) were obtained after being oven dried for 24 hours at 600 C. Also the net assimilation rate, relative growth rate and absolute growth rate was determined.

Relative turgidity

To determine the relative turgidity, the fresh leaf was soaked in water in a petri-dish overnight and was weighed. Mathematically,

Relative turgidity $= Weight\ of\ leaf\ (soaked\ in\ water) - initial\ fresh\ weight$ x 100 ………………1

Net assimilation rate = $(W_2 - W_1 (\log_e A_2 - \log_e A_1)/(A_2 - A_1)$ $(T_2 - T_1)$ ………………………………………………………………….2

Relative growth rate = $\frac{\log_e W2 - \log_e W1}{T1 - T2}$ …………………………………………………………………………………………………3

Absolute growth rate = $\frac{W2 - W1}{T2 - T1}$ ……………………………………………………………………………………………………....4

Where: Fwt= Fresh weight, Dwt = Dry weight, Twt = Turgidity weight, T2 = Final time, T1 = Initial time, T2-T1 = Time interval between initial time and final time, A2 = Leaf area at T2, A1 = Leaf area at T1, W2 = Total dry weight at T2, W1 = Total dry weight at T1

Data analysis

Data collected were subjected to statistical Analysis of Variances on the general linear model of SAS Software (SAS institute, inc.1999). Least Significant Difference (LSD) was used to further separate the means that were significantly different.

III. Results

Effect of mycorrhizal inoculation on morphological and physiological parameters

Mycorrhizal inoculation had no significant effect *($p > 0.05$)* on the variables measured. However, seedling height, relative turgidity and net assimilation rate had their highest mean value as (12.51cm), (8.47g) and (0.12gwk-1) respectively in the absence of the inoculation with mycorrhizal (M0) while the leaf number, leaf area and absolute growth rate had highest mean value of (4.56), (6.83 cm^2) and (-1.99gwk-1) respectively with ectomycorrhizal inoculation (M1) while leaf number (4.56) and absolute growth rate(-1.99gwk-1) were significantly different ($p<0.05$). Also, collar diameter (3.73mm) and relative growth rate (-0.07gwk-1wk-1) were increased in soil amended with ectomycorrhizal (Table 1).

Effect of watering regime on morphological and physiological parameters

The results showed that was no significant effect of watering regime on the morphological parameters and physiological parameters of *Garcinia kola* seedlings (p>0.05). The study showed that seedlings watered daily had the highest growth in leaf number and plant height as value of 4.41 and 12.22cm respectively. However, collar diameter (3.83mm) and net assimilation rate (0.13gwk-1) had the highest effect at two days interval. Also, leaf area (6.77cm^2), relative turgidity (7.82g), relative growth rate (-0.10gwk-1wk-1) and absolute growth rate (-1.96gwk-1) had greater effect when watered once in a week (Table 2).

Interactive effects of mycorrhizal inoculation and watering regime on morphological and physiological parameters

The interactive effect of mycorrhizal inoculation and the watering regime had no significant effect ($p<0.05$). Seedling height(14.03cm) were not significantly different when watered once a week with no mycorrhizal inoculation(M_0W_2), leaf number (5.22) were not significantly different when watered once daily with ectomycorrhizal inoculation(M_1W_0), leaf area (6.92cm^2) were not significantly different when watered once in a week with ectomycorrhizal inoculation(M_1W_2) and collar diameter (3.95mm) were not significantly different when watered once in a week with endomycorrhizal inoculation(M_2W_2), while the net assimilation rate (0.21gwk-1), relative growth rate (0.10gwk-1wk-1) and absolute growth rate (0.97gwk-1) were not significantly different with no mycorrhizal inoculation and watering every other day (M_0W_1). The relative turgidity (9.46g) was not significant with no mycorrhizal inoculation and watering once in a week (M_0W_2), (Table 3).

IV. Discussion

Seedlings inoculated with no mycorrhizal (M_0) recorded greater plant height, relative turgidity, net assimilation rate; Seedlings inoculated with ectomycorrhizal (M_1) recorded greater leaf number, leaf area, absolute growth rate and seedlings inoculated with endo mycorrhizal (M_2) recorded greater collar diameter and relative growth rate. However, the result showed non significant (P>0.05) difference between on plants inoculated with either of the mycorrhizal type (M_1, and M_2) in terms of plants height, collar diameter, relative turgidity, net assimilation rate, relative growth rate and absolute growth rate. These findings support the observations made by Allen and Boosalis, (1993) on *A. auriculiformis, A. lebbeck* and *Leucocephala.* Jasper, *et al.*, (1989) also found that root infection with mycorrhizae increases plant productivity and drought tolerance by improving phosphorus uptake. Similarly, Levy *et al.*, (1983) and Read and Boyd, (1986) reported that mycorrhizal inoculation increased soil water extraction and root hydraulic conductivity. According to Smith and Read, (2008), ectomyorrhizal is beneficial for tree seedlings both in the moist and dry environment, the study revealed that the leaf number was significantly different in seedlings that were inoculated with ectomycorrhizal. The result revealed that there was no significant difference in ectomycorrhizal inoculated seedlings, endomycorrhizal inoculated seedlings and no mycorrhizal inoculated seedlings and this can be supported by Smith and Read (2008) that plants are selective in their mycorrhizal association and it may be that *Garcinia kola* is selective in its association with ectomycorrhizal and endomycorrhizal.

Subsequently, it was noted that Bitter kola also responded to several watering regimes. Among the three watering regimes studied, the general growth response and reaction to water stress suggested that the seedlings that were watered daily (W_0) recorded the highest growth in leaf number and plant height, seedlings watered every other day (W_1) has the highest values in collar diameter and net assimilation rate and seedlings watered once in a week (W_2) has highest value in leaf area, relative turgidity, relative growth rate and absolute growth rate. Plant species respond variably to water availability and different plant parts adapt differently to variable water stress conditions (Gbadamosi, 2014). The amount of chlorophyll present in the plant was observed to be enhanced in seedlings watered every other day. Oyun *et al.* (2010), opined that cell division, its elongation as well as chlorophyll formation depends on plant water availability. In the presence of chlorophyll which is a vital unit for photosynthesis, there is an increase in the production of carbohydrate and water being an essential element in its formation as well as transportation in plants (Oyun *et al.*, 2010). Cernac *et al.*, (2006) also reported that adequate water supply is essential for effective growth and development of African breadfruit seedlings.

The interactive effect of mycorrhizae and watering regime showed no significant effect on major morphological parameters and physiological parameters of *Garcinia kola* seedlings. Seedling height were not significantly different when watered once a week with no mycorrhizal inoculation, leaf number were not significantly different when watered once daily with ectomycorrhizal inoculation, leaf area were not significantly different when watered once in a week with ectomycorrhizal inoculation and collar diameter were not significantly different when watered once in a week with endomycorrhizal inoculation, while the net assimilation rate, relative growth rate and absolute growth rate were not significantly different with no mycorrhizal inoculation and watering every other day. The relative turgidity was not significant with no mycorrhizal inoculation and watering once in a week.

V. Conclusion

The study concludes that irrespective of watering regime *Garcinia kola* seedlings will perform better with or without mycorrhizal. As a water-loving plant, *Garcinia kola* seedlings will perform better when watered once daily to pot capacity in terms of leave number and plant height. It can be concluded that this plant species may be selective in its mycorrhizal association.

It is therefore recommended that *Garcinia kola* seedlings should be raised with no mycorrhizal and watering once daily because *G. kola* seedling is water loving species. For effective growth further studies should be conducted on the different soil media on the growth performance of *Garcinia kola.*

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Table 1: The effect of mycorrhiza inoculation on the morphological and physiological parameters of *Garcinia kola* seedlings.

Treatment	Plant Height (cm)	Collar Diameter (cm)	Leaf Number	Leaf area (cm^2)	Relative Turgidity (g)	Net Assimilation Rate (%)	Relative Growth Rate (%)	Absolute Growth Rate (%)
M_0	12.51^a	3.53^a	3.98^{ab}	6.51^a	8.47^a	0.1184a	-0.0174a	-0.2033a
M_1	12.05^a	3.61^a	4.56^a	6.83^a	6.14^a	0.0998a	-0.0166b	-1.9966b
M_2	11.79^a	3.73^a	3.36^b	6.44^a	7.33^a	0.0907a	-0.0687ab	-1.3177a

Mean values with the same superscript in each column are not significantly different (P>0.05). **LSD**
M_0 – no mycorrhiza, M_1 – ecto mycorrhiza, M_2–endo mycorrhiza.

Table 2: The effect of watering regime on the morphological and physiological parameters of *Garcinia kola* seedlings.

Treatment	Plant Height (cm)	Collar Diameter	Leaf Number (cm)	Leaf area (cm^2)	Relative Turgidity (g)	Net Assimilation Rate (%)	Relative Growth Rate (%)	Absolute Growth Rate (%)
W_0	12.22^a	3.43^b	4.41^a	6.21^a	6.52^a	0.0994a	-0.0952a	-0.8355a
W_1	11.97^a	3.83^a	3.90^a	6.65^a	6.49^a	0.1301a	-0.0589a	-0.7177a
W_2	12.15^a	3.61^a	3.58^a	6.69^a	6.77^a	0.0795a	-0.0981a	-1.9644a

Mean values with the same superscript in each column are not significantly different (P>0.05). **LSD**

W_0 – watering once daily, W_1 – watering once every two days, W_2–watering once in a week.

Table 3: The effect of mycorrhiza inoculation and watering regime on the morphological and physiological parameters of *Garcinia kola* seedlings.

Treatment	Plant Height (cm)	Collar Diameter (cm)	Leaf Number	Leaf area (cm²)	Relative Turgidity (g)	Net Assimilation Rate (%)	Relative Growth Rate (%)	Absolute Growth Rate (%)
M_0W_0	11.48[b]	3.39[a]	4.49[ab]	6.21[a]	7.99[b]	0.0939[ab]	-0.0380[b]	-0.2833[ab]
M_0W_1	12.03[ab]	3.93[a]	3.69[b]	6.99[a]	7.96[b]	0.2140[a]	0.0960[a]	0.9666[a]
M_0W_2	14.03[a]	3.27[a]	3.27[c]	6.63a	9.46[a]	0.0474[ab]	-0.1100[ab]	-1.2933[a]
M_1W_0	13.36[ab]	3.44[a]	5.22[a]	6.65[a]	6.42[c]	0.1106[a]	-0.1183[ab]	-1.1300[ab]
M_1W_1	11.43[b]	3.78[a]	4.63[ab]	6.90[a]	6.25[c]	0.0870[ab]	-0.1881[ab]	-2.4433[ab]
M_1W_2	11.35[b]	3.62[a]	3.83[b]	6.92[a]	5.74[c]	0.1019[a]	-0.1918[ab]	-.2.4166[ab]
M_2W_0	11.83[b]	3.47[a]	3.05[c]	6.69[a]	5.61[c]	0.0936[ab]	-0.1294[ab]	-1.0933[ab]
M_2W_1	12.45[ab]	3.77[a]	3.38[c]	5.88[ab]	8.11[ab]	0.0894[ab]	-0.0841[ab]	-0.6766[ab]
M_2W_2	11.08[b]	3.95a	3.63[c]	6.75[a]	8.25[ab]	0.0892[ab]	0.0075[a]	-2.1833[ab]

Mean values with the same superscript in each column are not significantly different ($P>0.05$). **LSD**
M_0 – No mycorrhizal, M_1 – Ectomycorrhizal, M_2–Endomycorrhizal, W_0 – Watering daily, W_1 – Watering every other day, W_2 – Watering once in a week

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AUTHORS

First Author – Hammed, R. A, Department of Forestry and Wildlife Management, Federal University of Agriculture, Abeokuta, Nigeria

Second Author – Aduradola A.M, Department of Forestry and Wildlife Management, Federal University of Agriculture, Abeokuta, Nigeria

Third Author – Atanda, T.A, Department of Forestry and Wildlife Management, Federal University of Agriculture, Abeokuta, Nigeria

Fourth Author – Osoba, A.E, Department of Forestry and Wildlife Management, Federal University of Agriculture, Abeokuta, Nigeria

Fifth Author – Lawal, O.J, Department of Forestry and Wildlife Management, Federal University of Agriculture, Abeokuta, Nigeria

Correspondence:atandatoyeeb@gmail.com; +234(0)8161779030