

# **Relative Growth Rate and Net Assimilation Rate of Black afa (Termianliaivorensis) seedlings grown with Arbuscular Mycorrhizal Fungi (AMF) under NaCl stress in Calabar, Nigeria.**

## **ABSTRACT**

**Aim:** To ascertain how four distinct AMF species affect the relative growth rate (RGR) and net assimilation rate (NAR) of *T. ivorensis* under NaCl stress.

**Study Location and Length:** Calabar is situated in southern Nigeria. The study was carried out from February to May, 2022

**Methodology:** The field procedure was a 9x3x6 completely randomized design with nine treatments and three duplicates, which were further replicated six (6) times, totaling 162 pots. The *T. ivorensis* young plants were induced using four different AM fungi species (*Glomus mossae*, *Glomus intraradices*, *Glomus occultum* and *Glomus etunicatum*) and predisposed to 75mM of NaCl stress. At six sequential harvests (2,4,8,10 and 12 weeks after emergence), dry biomass and AMF colonization were determined. relative growth rate and net assimilation rate were calculated using the dry weight data obtained at harvest (4,6,8,10 and 12 WAE).

**Results:** All the NaCl stressed young plants indisputably showed higher colonization of AMF in their roots than their unstressed replica. Young plants inoculated using *Glomus intraradices* (*Gi*) in fusion with NaCl (*GiNaCl*) had the maximum AMF root colonization of 98.35%, while 31.78% was the least in plants treated with *G. occultum* (*Go*) at 12 and 8 WAE, respectively. Plants treated with *Glomus occultum* revealed  $0.46\text{gg}^{-1}\text{wk}^{-1}$  as the highest RGR value and  $0.22\text{gg}^{-1}\text{wk}^{-1}$  was recorded in *Gm*, *GmNaCl* and *GoNaCl* as the least value at 12 WAE. Furthermore, *Glomus etunicatum* treated plants recorded the maximum significant ( $p \leq 0.05$ ) NAR of  $0.55\text{gg}^{-1}\text{wk}^{-1}$  and the least was  $0.31\text{gg}^{-1}\text{wk}^{-1}$  in seedlings inoculated with *G. occultum* under salt stress at 12 WAE.

**Conclusion:** The regeneration of afforestation and vegetation in Nigeria's coastal areas can be facilitated by inoculating with the right AMF, which can also increase the biomass production and accumulation of *T. ivorensis* seedlings.

**Keywords:** *Arbuscular mycorrhizal fungi*; Black afara (*Terminalia ivorensis*); *Relative growth rate*; *Net assimilation rate*; NaCl stress.

## INTRODUCTION

Coastal regions, which span the globe from tropical regions to the Arctic and Antarctic, which symbolize distinct ecosystems that can be found between land and the ocean (1). With a 2500–3500 mm yearly rainfall, the Calabar coastline region is situated in Nigeria's high tropical rainforest belt (2). Nevertheless, the effective completion of vegetation regeneration of nearby coastal areas has been hampered by the excessive salt accumulation, shallow water table, and frequent severe weather. The primary and severe constraint on the preservation and restoration of vegetation in this area is the salinity of the soil (3). Growing salt concentrations in the soil limit plant growth and development by decreasing the plant's ability to absorb water and negatively affecting osmotic balance, net photosynthetic rate, nutrient absorbance, stomatal conductance, metabolic processes, and hydraulic conductivity (4).

Obligate biotrophs known as arbuscular mycorrhizal fungus (AMF) form mutualistic symbiotic relationships with the roots of vascular plants (5). By selectively absorbing K rather than Na, AMFs are hypothesized to have a cushioning effect in salt-affected soils, hence lowering the salt load of plant cells (6). The buildup of betaines, sugars, and prolines (osmoprotectants), which also create a helpful water gradient in roots even at greater Na concentrations in soil solutions, facilitated osmotic adaptations in AMF-infected plants. Additionally, AMF improves food availability, ionic balance, water the impact of AMF (6) on the growth and salt tolerance of native tree species in Nigeria's southern coastal regions is, nevertheless, poorly understood.

Within the Combretaceae family, *Terminalia ivorensis* (A. chev) is a huge forest tree with deciduous leaves that grows widely throughout the world's tropical and subtropical areas. It is natively found in Eastern, Western, and Southern Africa in about fifty species (7). Timber from *T. ivorensis*, an economically significant native tree species is utilized in Cross River State, Nigeria, in construction, especially for plywood production, joinery, flooring, fine woodwork, building etc (8). Because *T. ivorensis* can grow well in local, somewhat salinized soil, it is a viable choice for the coastal afforestation project (8). Despite their many applications, *T. ivorensis* seedlings are not very likely to survive in the high salinity

soil of the nearby coastal areas, which limits their use for afforestation in this location (3).

As a result, the current investigation was carried out with the assumption that AMF may increase *T. ivorensis* seedlings' growth biomass and salt tolerance. The impact of four distinct AMF species; *Glomus occultum*, *Glomus mossae*, *Glomus intraradices*, and *Glomus etunicatum* on the net assimilation rate (NAR) and relative growth rate (RGR) of *T. ivorensis* under salt stress (75 mM NaCl) were investigated in a pot experiment carried out in a field.

## **2. MATERIALS AND METHODS**

### **2.1 Research Area.**

The 12-week field study took place at the University of Calabar in Cross River State, Nigeria, between February and May of 2022. At roughly 70 meters above sea level, Calabar is located in the agro-ecology of the tropical high rainforest of Nigeria's climate in the equatorial area (Latitudes 5000' and 5040'N, Longitudes 8004' and 8062'E) (2). With an average yearly temperature range of 22.20-38.20 degrees Celsius and a comparatively high range of humidity of 75 to 90%, it has a bimodal distribution of rainfall, with a range of 2500–3500 mm of rainfall per year.

### **2.2 Soil, AMF inocula, and plant seedlings**

The *T. ivorensis* seeds were acquired in January 2022 from the Calabar, Department of Forestry, Ministry of Agriculture, and pre-treated using Wardsworth's recommended procedures (7). The International Institute for Tropical Agriculture, Ibadan, gave four different AMF species; *Glomus etunicatum*, *Glomus intraradices*, *Glomus mossae*, and *Glomus occultum* of inocula made up of infected root fragments, mycelium, and spores. The soil sample was taken between 0 to 20 cm below the surface, and it was employed as a growth medium after being sterilized for two hours at 160 degrees Celsius (9,3).

### **2.3 Treatments, Inoculation, and Experimental Planning**

Nine kilograms of sterilized soil were placed into each of the 39 cm in diameter and 49 cm deep pot, before being set up on racks in an actual field. These were irrigated until completely soaked, then allowed to drain overnight. To create mycorrhizal plants, fifty grams (50g) of coarse inoculum was placed in pots, 3 cm

below the soil's surface, intended for AM fungi assigned pots (9). After thinning, seedlings with uniform heights were chosen one week following emergence (WAE). The treatments are made up of; control (without AMF and NaCl), *Glomus etunicatum*(Ge), stressed *Glomus etunicatum*(GeNaCl), *Glomus intraradices* (Gi), stressed *Glomus intraradices*(GiNaCl), *Glomus mossae* (Gm), stressed *Glomus mossae*(GmNaCl), *Glomus occultum* (Go) and stressed *Glomus occultum*(GoNaCl). Two weeks following seedlings emergence, differential salinization was started in order to prevent plants from experiencing osmotic shock (10). Once a week, 50 milliliters of a 75 mM NaCl solution per pot was used to irrigate the salt seedlings. A total of 162 pots were involved in the 9x3x6 completely randomized design experiment, which included nine (9) treatments with three (3) replicates, each of which was further reproduced six (6) times (according to the six harvesting periods).

#### 2.4 Destructive Harvest and Biomass Determination

Total plant biomass was destructively determined on six successive harvest dates (2,4,6,8, 10 and 12 weeks after emergence) After deeply soaking the soil to soften it, the seedlings were gently removed at each harvest. Each plant was divided into its stems, leaves and root. It was then put in a paper package with a distinct label, and it was oven dried at 70 degree Celsius until it reached a constant dry weight (1). The total dry weight of the plant was calculated by adding the dry weight of the stem, leaf, and root. The net assimilation rate (NAR) and relative growth rate (RGR) were then computed using the dry weight data that were acquired. At harvest and at 4, 6, 8, 10, and 12 WAE, RGR and NAR were noted as;

$$RGR = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} g g^{-1} w k^{-1}$$

Where, ln= Natural logarithm,  $W_2$  and  $W_1$ =total dry weight at successive harvest stages,  $t_2$  and  $t_1$  = time interval respectively and is expressed as g/g/week (11).

NAR; It was calculated as the change in total plant biomass per leaf weight and time (12)

$$NAR = \frac{2(W_2 - W_1)}{(t_2 - t_1)(LDW_2 + LDW_1)} g g^{-1} w k^{-1}$$

where  $LDW_2$  and  $LDW_1$ = leaf dry weight at successive harvest stages

#### 2.5 AMF Colonization

At the time of each harvest, fresh feeder root subsamples were collected in order to calculate the proportion of AMF root colonization. After being properly cleaned in distilled water, the feeder roots were fixed in 50% ethanol. Staining and clearing were done using Koske and Gemma's approach (13). We assessed the stained roots for AMF colonization using Giovannetti and Mosse's gridline intersect technique (14). On a gridline plate, stained roots were spread out and examined at x45 magnification using a dissecting microscope. The following formula was used to get the percentage of root colonization:

$$\text{Root colonization}(\%) = \frac{\text{Number of arbuscular mycorrhizal – positive segments}}{\text{Total number of segments examined}} \times 100$$

## 2.6 Statistical Analysis

The standard error was computed using mean values derived based on the repeated measurements (n). One-way analysis of variance (ANOVA) was utilized to ascertain mean differences, and Duncan's multiple range test was employed to separate the means at  $p \leq 0.05$ . The statistical program for social sciences (SPSS) (version 20.0 for Windows) was used to analyze all of the data (15).

## 3. RESULTS

### 3.1 Soil and colonization by AMF

Physically, the soil used for the experiment constituted of 9.0% silt, 78.7% sand, 1.86% organic matter and 12.3% clay. It had a pH of 5.69 and a sandy loam texture according to the physicochemical study. Chemical composition: 0.8 cmol/kg Mg, 1.40 cmol/kg Ca, 85.0 mg/kg accessible P, 0.11 cmol/kg K, and 0.08 mg/kg total nitrogen.

The roots of *T. ivorensis* control seedlings did not exhibit mycorrhizal colonization. With the exception of two weeks after emergence (2WAE), salt stress continuously and significantly ( $P \leq 0.05$ ) enhanced the percentage root AMF colonization (Table 1). Inoculating seedlings with *Glomus mossae* (*Gm*) produced the least mean value of 54.18%, while plants inoculated with *Glomus intraradices* (*Gi*) under NaCl stress, which produced the maximum percentage root colonization of 98.35% (Table 1).

Table 1

Impact of mycorrhizal inoculation on *T. ivorensis* seedlings' root AMF colonization percentage under salt stress (%)

Treatment	2WAE	4WAE	6WAE	8WAE	10WAE	12WAE
<i>Ge</i>	64.49±2.07 <sup>c</sup>	72.98±1.76 <sup>d</sup>	63.95±2.54 <sup>c</sup>	63.86±2.12 <sup>d</sup>	67.86±1.96 <sup>c</sup>	71.26±2.01 <sup>c</sup>
<i>GeNaCl</i>	68.08±1.51 <sup>c</sup>	91.09±1.02 <sup>f</sup>	96.59±0.89 <sup>e</sup>	96.14±0.59 <sup>g</sup>	93.03±0.56 <sup>e</sup>	94.16±1.03 <sup>f</sup>
<i>Gi</i>	52.68±3.61 <sup>b</sup>	53.29±3.48 <sup>b</sup>	41.41±3.32 <sup>a</sup>	37.77±3.62 <sup>b</sup>	53.94±3.82 <sup>b</sup>	54.67±3.69 <sup>a</sup>
<i>GiNaCl</i>	55.23±3.43 <sup>b</sup>	97.74±0.37 <sup>g</sup>	91.81±0.67 <sup>e</sup>	89.58±0.59 <sup>f</sup>	96.44±1.18 <sup>e</sup>	98.35±0.54 <sup>f</sup>
<i>Gm</i>	57.14±1.03 <sup>b</sup>	47.21±0.89 <sup>a</sup>	57.19±0.82 <sup>b</sup>	52.05±0.98 <sup>c</sup>	50.40±1.94 <sup>ab</sup>	54.18±0.89 <sup>a</sup>
<i>GmNaCl</i>	58.02±1.03 <sup>b</sup>	79.11±0.69 <sup>e</sup>	73.95±0.79 <sup>d</sup>	64.96±0.93 <sup>e</sup>	81.04±1.07 <sup>d</sup>	77.12±1.14 <sup>d</sup>
<i>Go</i>	42.86±0.00 <sup>a</sup>	43.87±0.77 <sup>a</sup>	39.06±0.62 <sup>a</sup>	31.78±0.50 <sup>a</sup>	45.69±0.74 <sup>a</sup>	54.24±2.45 <sup>a</sup>
<i>GoNaCl</i>	43.26±1.39 <sup>a</sup>	63.52±0.77 <sup>c</sup>	72.24±0.97 <sup>d</sup>	59.58±1.14 <sup>d</sup>	70.50±0.96 <sup>c</sup>	65.48±0.79 <sup>b</sup>

\* Means of three replicates ± "standard errors of mean (SEM) Duncan's multiple range test indicates that means within each column followed by different letters are substantially different at  $P \leq 0.05$

### 3.2 Destructive Harvest and Biomass Determination

Generally, relative growth rate progressively decreases with time in all treatments across table 2 (from 4-12 WAE). For all treatments, the relative growth rate (RGR) of seedlings at 6 and 12 WAE did not differ significantly ( $P \geq 0.05$ ). Furthermore, at 12WAE, seedlings injected with *G. occultum* showed the greatest RGR mean value of  $0.46 \text{gg}^{-1} \text{wk}^{-1}$  and the lowest value of  $0.22 \text{gg}^{-1} \text{wk}^{-1}$  in *Gm*, *GmNaCl* and *GoNaCl* (Table 2).

UNDER PEER REVIEW

TABLE 2

Impact of mycorrhizal inoculation on *Terminalia ivorensis* relative growth rate ( $\text{gg}^{-1} \text{wk}^{-1}$ ) under salt stress

Treatment	4WAE	6WAE	8WAE	10WAE	12WAE
Control	1.30±0.00 <sup>b</sup>	0.29±0.06 <sup>a</sup>	0.16±0.01 <sup>a</sup>	0.25±0.01 <sup>b</sup>	0.25±0.02 <sup>a</sup>
<i>Ge</i>	1.49±0.08 <sup>bc</sup>	0.43±0.06 <sup>a</sup>	0.48±0.02 <sup>f</sup>	0.37±0.01 <sup>c</sup>	0.24±0.00 <sup>a</sup>
<i>Ge</i> NaCl	1.39±0.09 <sup>bc</sup>	0.36±0.07 <sup>a</sup>	0.30±0.00 <sup>cd</sup>	0.32±0.00 <sup>c</sup>	0.27±0.02 <sup>a</sup>
<i>Gi</i>	1.63±0.04 <sup>c</sup>	0.37±0.00 <sup>a</sup>	0.38±0.03 <sup>e</sup>	0.31±0.01 <sup>c</sup>	0.29±0.08 <sup>a</sup>
<i>Gi</i> NaCl	1.32±0.04 <sup>c</sup>	0.28±0.02 <sup>a</sup>	0.21±0.01 <sup>ab</sup>	0.20±0.04 <sup>ab</sup>	0.33±0.06 <sup>a</sup>
<i>Gm</i>	0.67±0.09 <sup>a</sup>	0.38±0.04 <sup>a</sup>	0.30±0.03 <sup>cd</sup>	0.32±0.04 <sup>c</sup>	0.22±0.00 <sup>a</sup>
<i>Gm</i> NaCl	0.59±0.06 <sup>a</sup>	0.32±0.04 <sup>a</sup>	0.24±0.02 <sup>bc</sup>	0.25±0.02 <sup>b</sup>	0.22±0.02 <sup>a</sup>
<i>Go</i>	0.77±0.15 <sup>a</sup>	0.63±0.25 <sup>a</sup>	0.35±0.02 <sup>de</sup>	0.32±0.01 <sup>c</sup>	0.46±0.00 <sup>a</sup>
<i>Go</i> NaCl	0.67±0.17 <sup>a</sup>	0.28±0.03 <sup>a</sup>	0.24±0.04 <sup>bc</sup>	0.17±0.01 <sup>a</sup>	0.22±0.01 <sup>a</sup>

\* Means of three replicates ± "standard errors of mean (SEM) Duncan's multiple range test indicates that means within each column followed by different letters are substantially different at  $P \leq 0.05$

The net assimilation rate was consistently higher in seedlings inoculated with AMF than their stress counterparts at 4,6,8,10 and 12WAE, except in seedlings inoculated with *G.intraradices* and stressed at 4 and 12WAE (Table 3). Seedlings stressed with NaCl recorded a reduction in NAR in most cases at 6,8,10 and 12WAE,except in seedlings inoculated with *G.intraradices* at 12WAE(Table 3). Seedlings infected with *G. etunicatum* also showed the highest significant ( $p\leq 0.05$ ) results at 12WAE. In seedlings injected with *G. occultum* under salinity stress, the lowest NAR was 0.31g/g/wk, and the highest was 0.55g/g/wk (Table 3).

TABLE 3

Impact of mycorrhizal inoculation on *Terminalia ivorensis* net assimilation rate ( $\text{g g}^{-1} \text{wk}^{-1}$ ) under salt stress

Treatment	4WAE	6WAE	8WAE	10WAE	12WAE
Control	1.76±0.00 <sup>c</sup>	0.64±0.10 <sup>ab</sup>	0.19±0.08 <sup>a</sup>	0.40±0.00 <sup>ab</sup>	0.46±0.02 <sup>bc</sup>
<i>Ge</i>	1.64±0.12 <sup>bc</sup>	0.88±0.05 <sup>b</sup>	0.73±0.03 <sup>e</sup>	0.69±0.00 <sup>ab</sup>	0.55±0.01 <sup>d</sup>
<i>Ge</i> NaCl	1.89±0.13 <sup>c</sup>	0.62±0.11 <sup>ab</sup>	0.45±0.01 <sup>cd</sup>	0.98±0.51 <sup>b</sup>	0.44±0.03 <sup>b</sup>
<i>Gi</i>	1.34±0.06 <sup>b</sup>	0.60±0.14 <sup>ab</sup>	0.56±0.02 <sup>d</sup>	0.59±0.02 <sup>ab</sup>	0.50±0.03 <sup>cd</sup>
<i>Gi</i> NaCl	1.35±0.05 <sup>b</sup>	0.37±0.20 <sup>a</sup>	0.28±0.02 <sup>ab</sup>	0.25±0.09 <sup>a</sup>	0.51±0.06 <sup>cd</sup>
<i>Gm</i>	0.82±0.08 <sup>a</sup>	0.52±0.05 <sup>ab</sup>	0.44±0.05 <sup>cd</sup>	0.76±0.05 <sup>ab</sup>	0.51±0.00 <sup>cd</sup>
<i>Gm</i> NaCl	0.75±0.04 <sup>a</sup>	0.45±0.03 <sup>a</sup>	0.33±0.02 <sup>bc</sup>	0.53±0.10 <sup>ab</sup>	0.41±0.04 <sup>b</sup>
<i>Go</i>	0.79±0.10 <sup>a</sup>	0.48±0.03 <sup>ab</sup>	0.49±0.02 <sup>d</sup>	0.58±0.05 <sup>ab</sup>	0.46±0.01 <sup>bc</sup>
<i>Go</i> NaCl	0.69±0.15 <sup>a</sup>	0.33±0.04 <sup>a</sup>	0.29±0.09 <sup>ab</sup>	0.19±0.04 <sup>a</sup>	0.31±0.00 <sup>a</sup>

\* Means of three replicates ± "standard errors of mean (SEM) Duncan's multiple range test indicates that means within each column followed by different letters are substantially different at  $P \leq 0.05$ .

#### 4. Discussion

Numerous abiotic stressors negatively impact plant growth, which lessens the encouragement of plant growth and explains the decline in agricultural productivity (16). Extreme salinity damages trees and has a negative impact on the composition of coastal forests and vegetation restoration initiatives (1) in Nigeria (3). On the other hand, AMF inoculation improves a plant's ability to withstand salt by altering physiological and biochemical processes, including enhanced growth, development, and metabolism (6).

Because *T. ivorensis* control seedlings were not mycorrhizal, there was no AMF colonization. The potential of the AMF to colonize is influenced by plant genotypes, host specialization, nutrient availability, and fluctuations in nutrient uptake under different environmental conditions (17). The findings of Chandrasekaram's (17) report, which indicate that the strains of a fungus influence symbiotic efficiency in addition to the host species and environmental factors, align with the differences observed in the current study's colonization patterns and the AMF between the various strains' reactions to NaCl stress. Additionally, the current study's findings show that *T. ivorensis* seedlings that were inoculated under NaCl stress had larger percentages of AM fungi colonization than plants that were not affected. These results are consistent with reports by (18 and 3) that show a significant increase in the percentage of AM fungi colonization in *Pterocarpus mildbraedii* and *Terminalia ivorensis* seedlings under 75mM NaCl stress. This implies that sporulation, which lengthens the hyphal and branching absorbing structures (BAS) of fungus, may be stimulated by salt stress (19). This contradicts the results of Wang *et al.* (1), who found that AMF hyphal development, sporulation, and spore germination were suppressed, leading to a decrease in the percentage of AM fungi colonization of *Zelkova serrata* plants subjected to salt stress.

Plant growth rate is measured using a metric called relative growth rate, or RGR. It calculates the rise in dry matter at a specific time with a specific amount of assimilatory material (20). A plant's RGR is influenced by a variety of plant characteristics, such as biomass allocation, germination time, and life history (21). Relative growth rate (RGR) is a common metric used to describe growth. However, the steady decline in the relative growth rate over time (4 to 12 WAE) observed in this study is consistent with the findings of Rees *et al.*, (22). He discovered that as size increased, RGR decreased and that physiology, morphology, and allocation

underwent systematic modifications. The RGR may decrease over time due to accelerating resource constraints (e.g., self-shading or soil resource depletion). Additionally, RGR frequently decreases over time as plant biomass rises (23, 24). In the meantime, Osim (3) found that the *T. ivorensis* seedlings' leaf tissues had a higher biomass allocation than their root or stem tissues, suggesting that the leaf serves as the main sink. However, because of the high dry mass of the leaves, there is a significant carbon loss through leaf respiration, which lowers the RGR significantly.

A helpful metric for assessing a plant's photosynthetic efficiency is the net assimilation rate, or NAR. It has to do with how quickly the dry weight of the entire plant increases per unit of leaf area and unit of time, which is related to the photosynthetic activities of the leaf. Expressed per unit leaf area, it is primarily the net consequence of carbon gains (photosynthesis) and losses (exudation, respiration, and volatilization). NAR was determined in this study by calculating the variation in total plant biomass per leaf weight and time (12). The significant variation in NAR found in this work is consistent with Chango and Velly's (25), which found that the rate of photosynthesis in siliquae increases and in leaves decreases as crops mature (i.e., at the siliquae production stage). This could be explained by the varying pace at which photoassimilate is supplied to the plant depending on its stage of vegetative growth as a result of siliquae production (25). Concurrently, the current study of RGR and NAR reductions of *T. ivorensis* seedlings under salt stress are comparable to Downton's findings (26). He discovered that at 75 mM NaCl, the concentration of carbohydrates in grapevine leaves decreased by 20–40%. This indicated that the decreased growth was caused by a decrease in photosynthesis, which was the consequence of a decrease in chlorophyll content and CO<sub>2</sub> assimilation (27). Moreover, Juniper and Abbott (28) documented the direct impact of NaCl on the fungal plant species, which led to a drop in NAR as a result of a rise in respiration rate (carbon loss) and energy expenditure. This is in contrast to the results of several other studies (29, 30, 31), who found that AMF-inoculated plants under salt stress had higher NAR. These data show that salt lessens the interference that salt causes with the production of chlorophyll in mycorrhizal plants, which promotes photosynthesis (29). AMF play a crucial role in biofortification of afforestation, and vegetation restoration of coastal regions. Application of AMF is a rather economical technique.

## 5 Conclusion

The coordinated operation of interdependent stems, leaves, and (mycorrhizal) roots determines how well a tree performs. The method accurately determines that net assimilation rate (NAR) is closely connected with relative growth rate (RGR) since RGR decreases with time due to increased resource constraint (as in the loss of soil resources or self-shading). During the course of the growth cycle, individuals are destructively sampled regularly. Applying a traditional growth study to successive harvests will yield the same results. Since age and size are interchangeable, all strategies are therefore equally legitimate. AMF has been applied to saline soil as a bioameliorator. In Calabar, Nigeria's salty sandy loam soil, inoculating seedlings of *T. ivorensis* with the right AMF can increase biomass production and seedling accumulation. As a result, it is advised that *G. mossae*, the most promising contender, be used to restore afforestation and vegetation in Nigeria's coastal areas.

## REFERENCES

- 1 Wang J, Fu Z, Ren Q, Zhu L, Lin J, Zhang J, Cheng X, Ma J, and Yue J. *Effect of Arbuscular mycorrhizal Fungi on Growth, Photosynthesis and Nutrient Uptake of Zelkova serrata* (Thunb) Makino Seedlings under salts stress. *Forest*. 2019;10(186): 1-16.
- 2 Iwena O. A. *Essential geography*. Ibanda: Tonal Publishers; 2008.
- 3 Osim S.E. Dry biomass accumulation of *Terminalia ivorensis* (A. Chev) seedlings inoculated with arbuscular mycorrhizal fungi under salt stress. *Journal of Science, Engineering and Technology*. 2021;8(1):10-17.
- 4 Abdel-Fattah, G.M, Rabie, G.H., Lamis, D.S. and Ralab, A.M. The Impact of the *Arbuscular mycorrhizal* Fungi on growth and physiological parameters of cowpea plants grown under salt stress conditions. *International Journal of Applied Sciences and Biotechnology*. 2016;4(3):372-379.
- 5 Chandrasekaran, M. Boopathi, T and Manivannan, p. Comprehensive assessment of ameliorative effects of AMF in alleviating Abiotic stress in Tomato plants. *Journal of Fungi*. 2021;7,303.
- 6 Khaliq A, Perveen S, Alamer KH, Haq MZU, Rafique Z, al Sudays IM, Althobaiti AT, Saleh MA, Hussain S, Attia H. *Arbuscular mycorrhizal*

- fungi* symbiosis to enhance plant-soil interaction. Sustainability. 2022;14:7840.
- 7 Wadsworth, F.H. Forest Production for Tropical America, Agricultural Handbook 710. United States Department of Agriculture. 1997.
  - 8 Lemmens RHMJ, Soerianegara I, Wong WC. Timber trees: Minor commercial timbers. Leiden: Backhuys Publishers; 1995.
  - 9 Rabie, G.H. & Almadini, A.M. Role of bio-inoculants in development of salt-tolerance of *Vicia faba* plants under salinity stress. King Faisal University, Saudi Arabia, *African Journal of Biotechnology*. 2005; 25: 221-242.
  - 10 Abbaspour, H., Fallahyan. F, & Fahimi, H. Effects of endo-mycorrhizal fungi and salt stress on nutrient acquisition and growth of *Pistacia vera* L. Asian Network. *Pakistan Journal of Biotechnology Sciences*, 2005; 8(7):1006-1010.
  - 11 Hunt, R. Plant growth and Analysis Studies in Biology-Number 96. Edward Arnold publishing, London, UK. 1978.
  - 12 Heilmeyer, H., Scholze, E.D. and Whale., D.M. Carbon and Nitrogen Partitioning in the Biennial Monocarp *Arctium tomentosum*. *Mill oecologia*, 1986;70:466-474.
  - 13 Koske R.E, Gemma J.N. A modified procedure for staining roots to detect V. A. mycorrhizas. *Mycology Research*. 1989;(92):486-488.
  - 14 Giovannetti, M. & Mosse. An evaluation of techniques for measuring vesicular *Arbuscular mycorrhizal* infection in roots. *New Phytologist*. 1980;84:489-500.
  - 15 AOAC. Association of Official Analytical Chemist. Methods of Analyss, Washington, DC, USA; 2003.
  - 16 Malhi, G.S., Kaur, M., Kaushik, P., Alyemini, M. N., Alsahli, A.A. and Ahmad, P. *Arbuscular mycorrhiza* in combating abiotic stresses in vegetables: An ecofriendly approach. *Saudi J. Bio. Sci.* 2021;28, 1465.
  - 17 Chandrasekaran M. *Arbuscular mycorrhizal* fungi mediated enhanced biomass, root morphology traits and nutrient uptake under drought stress: A meta-analysis. *Fungi*, 2022;8;660.
  - 18 Osim S. E., Etukudo M. M. Growth Response of *Pterocarpus mildbraedii* (Harms) seedlings inoculated with *Arbuscular mycorrhizal* fungi (AMF) under salt stress in Calabar, Nigeria. *Asian Journal of Research in Botany*. 2022;8(2):21-28.

- 19 Jahromi F, Aroca R, Porcel R, Ruizlozano J. M. Influence of salinity on the invitro development of *Glomus intraradices* and on the invivophysiological and molecular responses of mycorrhizal lettuce plants. *Microbial Ecology*. 2008;55:45-53.
- 20 Rajput, A., Rajput, S.S. and Jha, G. Physiological parameters leaf area index, crop growth rate, relative growth rate and net assimilation rate of different varieties of rice grown under different planting geometries and depths in SRI. *Int. J. Pure App. Biosci*. 2017;5(1): 362-367.
- 21 Fu, H., Yuan, G., Cao, T., Ni, L., Li., W. and Zhu, G. Relationships between relative growth rate and its components across II submerged macrophytes. *Journal of Freshwater ecology*. 2012;27(4),471-480.
- 22 Rees, M., Osborne, C. P., Woodward, F.I., Hulme, S.P., Turnbull, L. A. and Taylor, S. H. Partitioning the components of relative growth rate: How important is plant size variation? *The American Naturalist*. 2010;176:152-161.
- 23 Paine, C.E.T., Marthews, T. R., Vogt, D.R., Purves, D., Rees, M., Hector, A and Turnbull, L.A. “How to fit non-linear plant growth models and calculate growth rate: An update for Ecologist” *Methods in Ecology and Evolution*. 2012;3(3):245.
- 24 Christopher, D., Philipson, S., Toby, R., Marthew, T. R., Reuben, N., Glen, R., Lindsay, A., Turnbull, L. A. and Hector, A. Light based regeneration niches: evidence from 21 *Dipterocarp* spp using size-specific relative growth rates. *Biotropica*. 2012;3(4):265.
- 25 Chango, G. MC. And Velly, P.B.E. Relationship of physiological characters to yield parameters in oil seed rape. *Canadian Journal of Plant Science*. 2001;81:1-6.
- 26 Downton, W.J.S. Photosynthesis in salt stressed grapevines. *Aust.J. plant physio*. 1974;183-192.
- 27 Singh, M. and Singh, S. Net assimilation rate, relative growth rate and yield of pea genotypes under different NaHco<sub>3</sub> concentrations. *Biologia plantarum*. 1994;36(1): 145-148.
- 28 Juniper, S. & Abbott, L.K. Soil salinity delays germination and limits growth of hyphae form propagules of *Arbuscular mycorrhizal* fungi. *Mycorrhiza*, 2006;16:371-379.
- 29 Giri, B. and Mukerji, K. G Mycorrhizal inoculants alleviates salt stress in *Sesbania aegyptiaca* and *Sesbania grandiflora* under field conditions:

- Evidence for reduced sodium and improved magnesium uptake. *Mycorrhizal.*, 2004; 14:307-312.
- 30 Sheng, M., Tang., M., Chan, H., Yang, B., Zhang, F. and Huang, Y. Influence of *Arbuscular mycorrhizae* on photosynthesis and water status of maize plants under salt stress. *Mycorrhiza*. 2008;18:287-295.
- 31 Zuccarini, P. Mycorrhizal infection ameliorates chlorophyll content and nutrient uptake of lettuce exposed to saline irrigation. *Plant, Soil and Environment*. 2007;53:283-289.

UNDER PEER REVIEW