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Influence of Arbuscular Mycorrhizal Fungi on performance of *Amarathus viridis* cultivated in water-stressed Soil

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Abstract

Water scarcity has adverse effects on the nutrient supply of plants, reducing phosphate availability and exerts significant losses both in crop yield and quality. A pot experiment was conducted to investigate the influence of mycorrhiza on the growth of water-stressed Amaranthus viridis. Seeds of Amaranthus viridis were raised in the nursery and transplanted in the pots. Three species of mycorrhiza namely: Gigaspora gigantea, Glomus clarum and Glomus mossea were inoculated into the pot followed by the application of 5 levels (1 FC, 0.8 FC, 0.6 FC, 0.4 FC and 0.2 FC) of irrigation. The experiment was laid out in a Completely Randomized design. Plant growth parameters were taken 5 weeks after transplanting, while soil physico-chemical parameters were analyzed before and after the experiment using standard procedures. The results obtained showed that the Available Phosphorus in the Soil before planting (SBP) recorded 16.50 mg/kg but increased to 29.50 mg/kg on plants inoculated with mycorrhiza. The pH value of SBP was 4.20 but increased to 5.18 with mycorrhiza inoculation after harvest. At 5 weeks after transplanting, 1FC (Control) produced the highest plant height value, 4.13 cm but not significantly different from other plants with water-stressed plants (0.80 FC – 0.20 FC). Also, greater number of leaves were recorded in plants inoculated with G.gigantea at 0.2 FC (9.33 cm) and G.clarium at 0.4 FC (7.67 cm) and were significantly higher than plants inoculated with G.gigantea at 1 FC and G. mossea at 1 FC, respectively. The inoculation of Mycorrhiza on soils helped to ameliorate the water stress on the plant and enhanced the absorption and utilization of nutrient elements, particularly Phosphorus. Based on comparative assessment of the 3 species of Mycorrhiza used at various levels of irrigation; the Gigaspora gigantea at 0.2 FC outperformed others both in plant parameters and in soil nutrient elements. Therefore, farmers should be encouraged to adopt this method to cushion the deleterious effect of climate change on Amaranthus viridis.

Keywords: *Mycorrhiza, water scarcity, Irrigation, Field capacity, Amaranthus.*

Introduction

Traditional leafy vegetables (TLVs), like *Amaranthus*, have been vital to rural household food systems in Africa for generations, particularly among low-income populations in tropical regions such as Nigeria [19]. The importance of *Amaranthus* as a vegetable cannot be overstated. Its leaves and tender shoots are commonly boiled and prepared with modern culinary ingredients and they may also be dried during the dry season for use. *Amaranthus* is one of the few dicotyledonous plants that exhibit C_4 photosynthetic metabolism, a highly efficient photosynthetic pathway that confers high productivity. This characteristic makes *Amaranthus* a valuable vegetable crop for enhancing food and nutrition in developing African countries [12].

Water scarcity threatens not only arid and semi-arid regions but also other agricultural productive areas that depend on adequate water availability for successful horticulture. Ongoing climate change is expected to intensify both the frequency and severity of drought events worldwide [17], possibly undermining agricultural success achieved to date. Drought represents one of the most severe abiotic stresses, causing greater reductions in crop productivity than most other stress factors [11]. Limited water availability induces stomatal closure, which restricts CO_2 uptake and later reduces photosynthetic activity and carbon allocation [15]. In addition, water stress adversely affects nutrient availability, particularly phosphorus. Severe drought conditions adversely impact plant physiology, growth, development, and reproduction, leading to substantial yield losses and reduced crop quality. Thus, there is an urgent need to develop strategies that could enhance agricultural resilience and mitigate the adverse effects of water scarcity on crop productivity. Such strategies include increased attention to beneficial soil microorganisms, particularly arbuscular mycorrhizal (AM) fungi. Arbuscular mycorrhizal fungi are ubiquitous soil microorganisms capable of forming symbiotic associations with the majority of terrestrial plants. These fungi provide numerous benefits to their host plants [4]. Beyond improving plant nutritional status, AM fungi enhance plant performance and tolerance to various environmental stresses, especially drought stress. The utilization of AM fungi is considered one of the most effective approaches for increasing plant tolerance to environmental stressors [3].

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Previous studies have demonstrated that AM symbiosis significantly enhances plant tolerance to water deficit through improved water and nutrient uptake, modifications in host physiology such as photosynthesis and osmotic adjustment, regulation of phytohormones, and the activation of more efficient antioxidant defense systems [7]. Regrettably, there is still dearth of information on the comparative effect of mycorrhiza inocula on the performance of Amaranthus under varying water-stressed environments. Hence, this study is a necessity. Therefore, the objectives of this study were to:

I. ascertain the comparative effect of mycorrhizal inocula on the performance of *Amaranthus viridis* under 5 levels water stress.

ii. identify the best host variety for AMF multiplication under the prevalent soil condition

Materials and Methods

Study area

The experiment was conducted at the Screen house of the Department of Crop and Soil Science, University of Port Harcourt at latitude $4^{0}54$ N and longitude $06^{0}55$ E with an average temperature of 27 ^{0}C, relatively humidity of 78 % but decreases slightly in dry season and an average rainfall ranging from 2500 – 4000mm per annum [2]. The area has a bimodal rainfall pattern with a long rainy season usually between March and July and a short rainy season from September to early November after a short dry spell in August and a longer period from December to February [1].

Soil Sampling and data collection

Samples of soil (0 to 30 cm depth) were taken randomly from the research and teaching farm for sterilization. The soil collected was sterilized at a temperature of 121 ^{o}C for 4 hours. Data on plant growth parameters collected at 1-week interval were plant height (cm). Number of leaves. Leaf area (cm) and stem girth (cm).

Source of Amaranthus spp

The *Amaranthus spp* used for the experiment was gotten from Rivers State Agricultural Development Programme (ADP), Port Harcourt.

mycorrhiza fungi and source

The source of the mycorrhiza is from the Department of Microbiology, University of Ibadan. AMF inoculum: pure strains of 3 species of AMF were used for the experiment, namely *Glomus. clarium, Gigaspora. gigantea* and *Glomus. mossea*.

Design and Treatments

The experiment consisted of two factors: three species of mycorrhiza and five irrigation levels, arranged in a Completely Randomized Design (CRD). Amaranth seeds were sown in cell trays and allowed to germinate, after which the seedlings were maintained in the trays for six weeks. Prior to transplanting, pure strains of arbuscular mycorrhizal fungi (AMF) were inoculated into the experimental pots at a rate of 20 g per pot. One amaranth seedling was transplanted into each plastic pot with an internal bottom diameter of 30 cm, an internal top diameter of 30 cm, and a height of 35 cm. The five irrigation treatments consisted of 20% field capacity (0.20 FC), 40% field capacity (0.40 FC), 60% field capacity (0.60 FC), 80% field capacity (0.80 FC), and 100% field capacity (1.00 FC). Irrigation levels were monitored using tensiometers (Irrometer Co., Riverside, California, USA) by measuring soil water potential. One tensiometer was installed in a representative pot for each treatment at a soil depth of 10 cm to guide irrigation scheduling. Irrigation was applied whenever soil water potential reached −20 kPa (centibars), with watering carried out at three-day intervals. Field capacity was determined using the gravimetric method. At field capacity, the volume of water required per pot was 27 cl. Accordingly, irrigation treatments of 0.20 FC, 0.40 FC, 0.60 FC, 0.80 FC, and 1.00 FC corresponded to 5.4 cl, 11 cl, 16 cl, 22 cl, and 27 cl of water per pot, respectively.

Agronomics practices

Cultural practices were observed throughout the period of the experiment. Weeding was done manually using the handpicking method. Watering was done once at an interval of 3 days in the morning or evening.

Collection of Data

The following data were collected, number of leaves, plant height, stem girth and leaf area. The first data collections were done two weeks after transplanting (WAT). Thereafter, data were collected at an interval of one week.

Laboratory analysis

Particles size distribution was done using the hydrometer method as described by [5]. Soil pH was determined in 1:1 (soil: water) ratio using a glass electrode pH meter. Organic carbon was determined by the wet oxidation method [20]. Total nitrogen was by the micro Kjeldahl digestion method. Available phosphorus was determined by Bray 1 method [6]. Sodium and K were determined with a flame photometer while Ca and Mg were determined with the atomic absorption spectrophotometer (AAS). It is worthy to note that Soil before planting (SBP) and after planting were analyzed at the end of the experiment.

Data analysis

Data were analyzed using Gen Stat Software (GEN, 2012) and means separated using least significance difference (LSD) at 5% significance level.

Results

Effect of Mycorrhiza and 5 levels of irrigation on Plant Height of *Amaranthus viridis*

Table 1 shows the influence of AMF and different levels of water application on plant height of *amaranthus*. The plant inoculated with *G.gigantea* at 0.2 FC and G. mossea at 1 FC recorded the highest plant height, 4.13cm respectively. Among plant inoculated with G. gigantea, 0.2 FC irrigation level recorded the highest plant 4.13 cm and it is significantly higher than values obtained at other levels of irrigation. However, there was no significant difference among the other levels of irrigation. Among the plant inoculated with *G. clarium*, 0.4 FC recorded the highest plant height and is significantly different from others while 1 FC, 0.8 FC, 0.6 FC, and 0.2 FC showed no significant difference among them. Among the plant inoculated with *G. mossea*, the highest plant height was recorded at 1 FC level of water application and is highly significant form other levels of irrigation while 0.8 FC, 0.6 FC, 0.4 FC, and 0.2 FC showed no significant difference. The interaction effect between G. mossea (4.13) at 1 FC and G. clarium at 0.8 FC showed that there is no interaction between them also the interaction between G. gigantea at 0.2 FC and G. clarium at 0.4 FC showed no interaction between them.

Table 1: Effect of Mycorrhiza and 5 levels of irrigation on plant height of Amaranthus viridis at 5 WAT.

AMF spp		← levels of applied Irrigation →				
	Mean	1 FC	0.8 FC	0.6 FC	0.4 FC	0.2 FC
G. deserticola	3.11	2.60	3.17	3.03	2.63	4.13
G. gigantea	2.85	2.43	3.10	2.70	3.20	2.85
G. mossea	2.98	4.13	2.60	2.70	2.80	2.98
LSD (S)	0.811					
LSD (I)	1.047					
LSD (S X I)	1.813					

S = Species, W = levels of applied Irrigation applied, S X I = interaction of Specie and irrigation applied. LSD at 5 % probability level, WAT = weeks after planting.

Effects of Mycorrhiza and 5 levels of irrigation on Leaf Area of *Amaranthus viridis*

Table 2 shows the influence of AMF and different levels of water on the Leaf Area of Amaranthus. Plant inoculated with *G. mossea* at 1 FC level of water recorded the highest leaf area, 2.01 cm^2 and significantly higher than other values. Among plants inoculated with *G. gigantea,* 0.2 FC level of irrigation recorded the highest value of leaf area, 1.64 cm^2 and is significantly from other levels of irrigation. However, there was no significant difference among 1 FC, 0.8 FC, 0.6 FC, and 0.4 FC, respectively. Among the plant inoculated with *G. clarium,* 0.2 FC recorded the highest value of leaf area 2.01 cm^2 and was significantly different 1 FC, 0.8 FC, 0.6 FC, and 0.4 FC showed no significant difference among them. Among the plant inoculated with *G. mossea,* 1 FC level of irrigation recorded and is highly significant form other levels of water application while 0.8 FC, 0.6 FC, 0.4 FC and 0.2 FC has no significant difference. The interaction effect between *G. mossea* 2.01 cm^2 at 1 FC and *G. clarium* at 0.8 FC showed that there is no interaction between them also the interaction between G. gigantea at 0.2 FC and *G. clarium* at 0.4 FC showed no interaction between them.

Table 2: Effect of Mycorrhiza and 5 levels of irrigation on Leaf Area of Amaranthus viridis at 5 WAT

AMF spp		← levels of applied Irrigation →				
	Mean	1 FC	0.8 FC	0.6 FC	0.4 FC	0.2 FC
G. deserticola	0.92	0.54	1.00	0.56	0.85	1.64
G. gigantea	0.72	0.50	0.80	0.79	0.66	0.87
G. mossea	0.90	2.01	0.49	0.50	0.72	0.77
LSD (S)	0.651					
LSD (I)	0.840					
LSD (S X I)	1.455					

S = Species, W = levels of applied Irrigation applied, S X I = interaction of Specie and irrigation applied. LSD at 5 % probability level, WAT = weeks after planting.

Effect of Mycorrhiza and 5 levels of irrigation on stem girth of *Amaranthus viridis*

Table 3 shows the influence of AMF and different levels of irrigation on the stem girth of Amaranthus, plants inoculated with *G mossea* at 1 FC level of irrigation and *G. gigantea* recorded the highest stem girth, 0.74 cm, respectively. Among plant inoculated with *G. gigantea,* 0.2 FC level of irrigation recorded the highest stem girth, 0.74 cm. however has no significant difference among other level of irrigation 1 FC, 0.8 FC, 0.6 FC, and 0.4 FC, respectively. Among the plant inoculated with *G. clarium* 0.2 FC and 0.4 FC level of irrigation recorded the highest 0.64 cm stem girth, respectively and was significantly higher than other levels of irrigation. Among the plant inoculated with *G. mossea* the highest stem girth was recorded at 1 FC 0.74 cm level of irrigation and was significantly higher than other levels of irrigation –; however, 0.8 FC, 0.6 FC, 0.4 FC, and 0.2 FC has no significant difference respectively. The interaction effect between *G. mossea* at 1 FC and *G. clarium* at 0.8 FC showed that there was no interaction between them. Also, the interaction effect between *G. gigantea* at 0.2 FC and *G. clarium* at 0.4 FC showed no interaction.

Table 3: Effect of Mycorrhiza and 5 levels of irrigation on stem girth of Amaranthus viridis at 5 WAT

AMF spp		← levels of applied Irrigation →				
	Mean	1 FC	0.8 FC	0.6 FC	0.4 FC	0.2 FC
G. deserticola	0.62	0.54	0.64	0.60	0.60	0.74
G. gigantea	0.59	0.57	0.60	0.50	0.64	0.64
G. mossea	0.56	0.74	0.54	0.40	0.60	0.54
LSD (S)	0.154					
LSD (I)	0.199					
LSD (S X I)	0.345					

S = Species, W = levels of applied Irrigation applied, S X I = interaction of Specie and irrigation applied. LSD at 5 % probability level, WAT = weeks after planting.

Effect of Mycorrhiza and 5 levels of irrigation on number of leaves *Amaranthus viridis.*

Table 4 shows the influence of AMF and a different level of irrigation on number of leaves of Amaranthus, G. gigantea at 0.2 FC level of irrigation recorded the highest leave number 9.33 and is highly significant from others. Among plant inoculated with *G. gigantea,* 0.2 FC level of irrigation recorded the highest number of leaves, 9.33 and was significantly higher than other level of irrigation. However, plants at 1 FC, 0.8 FC, 0.6 FC, and 0.4 FC recorded no significant difference respectively. Among the plant inoculated with *G. clarium,* 0.4 FC level of irrigation recorded the highest number of leave 7.67 and was significantly higher from other levels of irrigation respectively. Among the plant inoculated with *G. mossea,* the highest number of leave – 7.33 was recorded at 1 FC and 0.2 FC level of irrigation respectively and was significant difference than other levels. However, other level of irrigation: 0.8 FC, 0.6 FC, and 0.4FC and 0.2 FC showed no significant difference. The interaction effect between *G. mossea* at 1 FC and *G. clarium* at 0.8 FC showed no interaction between them. Also, the interaction between *G. gigantea* at 0.2 FC and *G. clarium* at 0.4 FC showed no interaction.

Table 4: Effects of Mycorrhiza and 5 levels of irrigation on number of leaves of Amaranthus viridis at 5 WAT.

AMF spp		← levels of applied Irrigation →				
	Mean	1 FC	0.8 FC	0.6 FC	0.4 FC	0.2 FC
G. deserticola	7.47	6.67	7.00	7.00	7.33	9.33
G. gigantea	6.87	7.00	7.00	6.00	7.67	6.67
G. mossea	6.87	7.33	6.67	6.33	6.67	7.33
LSD (S)	1.268					
LSD (I)	1.636					
LSD (S X I)	2.834					

S = Species, W = levels of applied Irrigation applied, S X I = interaction of Specie and irrigation applied. LSD at 5 % probability level, WAT = weeks after planting.

Soil Physicochemical properties

Result showed that the AMF inoculated soil recorded higher pH values with range, 4.40 – 5.18 than the pH value obtained on SBP. Among plants inoculated, *G. gigantea,* at 0.2 FC of irrigation recorded the highest pH value, 5.18 while 0.6 FC recorded the least pH value of 4.45. Plants inoculated with *G. clarium* at 1 FC of irrigation (Control) recorded a higher value of 4.88 while 0.4 FC recorded the least value of 4.50. Plants inoculated with *G.mossea* at 1 FC of irrigation recorded a higher value of 4.78 while 0.6 FC recorded the least value of 4.40. The SPB had a clay value of 13.40 %. Among plants inoculated G. *gigantea,* at 1 FC and 0.8 FC recorded the highest value of 13.40 respectively while 0.6 FC, 0.4 FC and 0.2 FC recorded the least value of 11.40 respectively.

Table 5: Soil Physicochemical properties

Samples	pH (H_2O)	Sand	Silt	Clay	TOC	TN	Avail P	Ca	Mg	K	Na
		→	%	←	mg/kg	%		→	cmol/kg	←	
SBP	4.20	69.80	16.80	13.40	0.79	0.084	16.50	4.00	2.80	0.122	0.121
M1L1	4.62	73.80	12.80	13.40	1.03	0.098	18.20	5.60	2.00	0.134	0.148
M1L2	4.86	73.80	12.80	13.40	1.43	0.140	29.50	4.40	2.40	0.118	0.165
M1L3	4.45	77.80	10.80	11.40	0.83	0.084	18.80	4.80	3.20	0.121	0.182
M1L4	4.63	73.80	14.80	11.40	1.03	0.112	23.20	3.60	2.80	0.116	0.148
M1L5	5.18	71.80	16.80	11.40	0.63	0.056	14.50	3.20	2.80	0.114	0.131
M2L1	4.88	77.80	9.80	12.40	0.99	0.098	21.00	4.00	3.20	0.126	0.157
M2L2	4.65	75.80	11.80	12.40	0.63	0.077	14.70	4.00	2.40	0.135	0.156
M2L3	4.70	77.80	12.80	9.40	1.31	0.140	28.40	4.40	2.40	0.130	0.113
M2L4	4.50	77.80	9.80	12.40	0.87	0.084	19.40	5.60	4.00	0.115	0.139
M2L5	4.70	79.80	7.80	12.40	1.11	0.126	24.60	4.00	2.80	0.127	0.165
M3L1	4.78	77.80	12.80	9.40	0.71	0.056	71.10	3.60	2.40	0.138	0.183
M3L2	4.43	77.80	9.80	12.40	1.28	0.140	25.00	3.60	2.00	0.134	0.147
M3L3	4.40	77.80	12.80	9.40	0.67	0.063	14.60	4.00	2.00	0.131	0.174
M3L4	4.70	73.80	13.80	12.40	1.40	0.154	25.30	2.80	1.60	0.123	0.130
M3L5	4.61	73.80	13.80	12.40	0.40	0.056	71.00	3.20	2.00	0.127	0.148

M1=Gigaspora gigantea; M2= Glomus clarium; M3 Glomus mossea, L1 – L5= levels of water where L1 is Control, SBP= Soil value before planting, TOC = Total Organic Carbon, Avail P = Available Phosphorus, TN= Total Nitrogen

Among the plants inoculated with G*. mossea,* at 0.8 FC, 0.4 FC and 0.2 FC recorded the highest value of 12.40 while 1 FC and 0.6 FC had the least value of 9.40 %. Silt had 16.80 in SBP but decreased in soil inoculated with AMF *G. gigantea*, *G clarium and G mosse*a respectively. Sand in SBP recorded a value of 69.80 % but increased in AMF inoculated soil, G. *gigantea* at 0.6 FC recorded the highest value of 77.80 %, while 0.2 FC recorded the least value of 71.80 %. The plants inoculated with *G.* clarium at 0.2 FC recorded the highest value of Sand, 79 .80 % while 0.8 FC recorded the least value of Sand, 75.80 %. The plants inoculated with *G. mossea* at 1 FC, 0.8 FC 0.6 FC recorded the highest value of 77.80 % while 0.4 FC and 0. 2 FC recorded the least value, 73.80 % respectively. There was an increase in phosphorus (P) in AMF inoculated soil, SBP had a P value of 16.50 mg/kg but this increased in *G. gigantea* at 0.8 FC to 29.50 mg/kg while 0.2 FC recorded the least value of 14.00 mg/kg. Plants inoculated with *G. clarium* had an increase in phosphorus at 1 FC, 0.6 FC, 0.4 FC, 0.2 FC but decreased in 0.8 FC level of irrigation. Plants inoculated with *G.mossea* also had an increase in phosphorus with 1FC, 0.8 FC, 0.4 FC of irrigation. Total Organic Carbon had a similar record, 0.79 % was recorded in SBP but increased in G. gigantean with 1 FC, 0.8 FC, and 0.6 FC but decreased to 0.63 % in 0.2 FC level of irrigation. The plants inoculated with *G. mossea* increased at 0.8 FC and 0.4 FC but decreased at 1 FC, 0.6 FC and 0.2 FC of irrigation. The SBP recorded TN value of 0.084 % and increased in G. gigantea with water at 1 FC, 0.8 FC, 0.4 FC but decreased to 0.056 % at 0.2 F. Plants inoculated with *G. clarium* had an increase in 1 FC, 0.6 FC and 0.2 FC but decreased in 0.8 FC. The plants with inoculation of *G. mossea* decreased to 0.056 % at 1 FC, 0.6 FC and 0.2 FC but increased at 0.8 FC and 0.4 FC level of irrigation.

Discussion

The initial soil analysis indicated that soil pH increased in treatments involving mycorrhizal inoculation under irrigation compared with the soil before planting (SBP). This observation is consistent with the findings of [10], who reported that soil amendment with mycorrhiza can modify soil physicochemical properties, resulting in increased soil pH and enhanced nutrient availability through root colonization by mycorrhizal fungi. Nutrient concentrations were higher in soils associated with mycorrhiza-inoculated plants than in SBP, supporting the report of [13], which demonstrated that mycorrhizal fungi enhance root efficiency for nutrient absorption in nutrient-depleted soils.

Soils inoculated with mycorrhiza also exhibited increased phosphorus content across different irrigation levels compared with SBP. This result aligns with earlier studies indicating that mycorrhizal fungi develop extensive hyphal networks in the soil, thereby facilitating phosphorus uptake beyond the root hair zone [3,4]. The observed improvements in growth parameters of *Amaranthus* inoculated with mycorrhiza under water-stressed conditions are in agreement with the findings of [9], who reported that arbuscular mycorrhizal fungi (AMF) positively influence plant growth and development. Similarly, [16] and [18] reported that AMF colonization of plant root systems enhances the ability of host plants to withstand water stress by improving nutrient and water uptake, leading to increased growth and yield. These findings suggest that mycorrhizal inoculation effectively mitigates the adverse effects of water stress on plants. Overall, AMF contribute to improved nitrogen acquisition, which promotes vegetative growth and increases the production of green leaves in *Amaranthus* [14].

Conclusion

Water stress exerts deleterious effect on growth and performance of *Amaranthus viridis*. However, the inoculation of mycorrhiza on soils could ameliorate the abiotic stress on plant. AMF also enhances the absorption and utilization of nutrient element particularly the phosphorus, thereby increasing the yield of *Amaranthus viridis*. Based on comparative assessment of the 3 species of mycorrhiza used at various levels of irrigation; *Gigaspora. gigantea* at 0.2 F performed better than the others both in plant parameters and in soil nutrient elements.

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