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Enhancement of Salt Tolerance via *Glomus geosporum* Inoculation in *Telfairia occidentalis* Hook. F. Seedlings

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Abstract. The leafy vegetable *Telfairia occidentalis* is a tropical vine grown in West Africa; it is indigenous to Southern Nigeria and is usually subjected to extreme salt stress in Southern Nigeria as well as in the world that results in significant loss of *T. occidentalis* production. Therefore, the present investigation was aimed at evaluating the response of *T. occidentalis* seedlings inoculated with arbuscular mycorrhizal fungi (*Glomus geosporum*) in saline soil and further to determine the threshold of *T. occidentalis* salinity tolerance in association with *G. geosporum*. The total photosynthetic pigments contents in saline soil treatment were significantly (p=0.05) reduced as well as percentage arbuscular mycorrhizal fungi colonization (53.97 to 22.41%). Mycorrhizal dependency was significantly (p=0.05) higher in saline soil treatments compared to control (100.00% to 15.13%). Mineral analysis of *T. occidentalis* leaves revealed increased uptake and accumulation of Na^+ (500.00 mg/kg in control to 2920.13 mg/kg in saline soil treatment). Saline soil treatments significantly (p=0.05) reduced the K, Mg, N, P and Ca. AM Fungi significantly (p=0.05) increased the photosynthetic pigments and minerals both in saline and non-saline soil treatments. Using different mechanisms *T. occidentalis* by association with *G. geosporum* showed better salt tolerance thank the uninoculated plants. *G. geosporum* was able to impose some physiological and root morphological changes such as an extensive network of the mycorrhizal-plant roots to improve water and mineral nutrient uptake. Physiologically *G. geosporum* inoculation enriched *T. occidentalis* vigour, attuned the rate of K^+/Na^+ which restored nutrient and water balance in the plant and directly resulting in the enhancement of salt tolerance in *T. occidentalis* seedlings, thus improving growth and yield.

Introduction

One of the most serious threats facing crop production globally today is the excess accumulation of soluble salts in the soil [1, 2]. High soil salinity levels are so rampant and according to an estimate; about 7% of global lands are affected by soil salinity [2]. When water-soluble salts, predominantly chloride, sodium, magnesium, potassium and calcium in the soil, the scenario is known as soil salinity. A saline soil is known when its electrical conductivity of a saturated paste extract (ECe) arrives at the value of 4 dS/m or higher (which is similar to equivalent to 40 mM NaCl) [3]. At this ECe, most crops yield is significantly reduced.

There are many important salts which are soluble in soil solutions and could be considered as a very good media for the growth and development of crops [4]. These salts which are needed by plants to carry out various metabolic activities are absorbed by plant roots and are translocated to the different plants parts which they are required. However, when these salts are present in excessive quantities in the soil, they hinder plant nutrient and water uptake which in turn disrupts the ion sharing at both the cellular and the whole-plant levels, thus prompting osmotic and ionic imbalances [4]. Changes of that magnitude usually results in reduced growth and development of plants, subsequently resulting in the whole plant death. Oxidative injury which is a secondary stress resulting from the excessive accumulation of salts like Na^+ and Cl^- in plant tissues. This oxidative injury affects plasma membrane integrity which is as a result of damage to membrane components

such as proteins, lipids and nucleic acids, weakening the actions of biocatalysts and operations of the photosynthetic apparatus, This upset in proper functioning can be attributed to the production of the harmful reactive oxygen species (ROS) which is generated during salt stress [5, 6, 4].

Beneficial microorganisms for example arbuscular mycorrhizal fungi (AMF) can colonize plants even at their natural habitat. Plant tolerance under stressed conditions as well as crop yield can be improved by inoculation of such plants with useful bacteria and fungi [7]. It is estimated that about 80% of land-dwelling plant species roots form association with arbuscular mycorrhizal fungi [8]. Many researchers have shown that arbuscular mycorrhizal fungi promote plant growth under salt stressed conditions. AM fungi have been shown to promote plant growth and salinity tolerance by many researchers. Mycorrhizal fungi are found in all ecosystems [2, 9]. The association of plant and mycorrhizas dates back to over 400 million years [2]. The level of dependency on mycorrhizas differs with plant species, for example faba bean (*Vicia faba*) is depends highly on mycorrhizas and their growth and establishment depends on highly on its fungal association [10].

T. occidentalis belongs to the family *Cucurbitaceae* and is a tropical vine grown in West Africa as a leafy vegetable and for its edible seeds. It is indigenous to Southern Nigeria [11]. The fluted pumpkin grows in many nations of West Africa, but is mainly cultivated in Nigeria, used primarily in soups and herbal medicines [12]. *T. occidentalis* is one of the most commonly used vegetable crops in Nigeria. The edible seeds can be boiled and eaten whole or fermented [13].The fluted gourd has been traditionally used by some indigenous tribes as a blood tonic, likely due to its high protein content [14].

Glomus geosporum belongs to the family *Glomeraceae*. Spores of *G. geosporum* are formed singly in the soil; yellow (3A8) to orange (5B8); globose to subglobose; (130) 175 (260) µm diameter; sometimes ovoid; 130-150 x 220-260 µm; with a single subtending hypha. The mycorrhizae of *G. Geosporum* consisted of arbuscules, vesicles, as well as intra- and extra radical hyphae. The arbuscules and vesicles were patchily distributed along the roots examined [15].

This research investigates the influence of AM fungi (*G. geosporum*) inoculation on *T. occidentalis* tolerance to salt stress.

Materials & Methods

Study Area

Saline soil and salt water were collected from the saline ecosystem of Iwuochang, Ibeno Local Government Area (Latitude 4.56ºN and Longitude 7.57ºE), Akwa Ibom State, Nigeria, with an annual rainfall of about 4021 mm and mean temperature variation of 22 – 31ºC. The experiment was set up in a safe and secured environment at Mbioto 1, Etinan Local Government Area (Latitude 4.51ºN and Longitude 7.50ºE), Akwa Ibom State, Nigeria, with an annual rainfall of about 4000 mm and mean temperature variation of 26 – 36ºC [16]. Non-saline soil for the control and non-saline treatments was obtained from a farmland in Mbioto 1, Etinan Local Government Area; fresh water was used for watering the non-saline and control treatments. A map showing the saline water/soil collection and experimental set-up locations is presented in Figure 1.

Source of Arbuscular Mycorrhizal (AM) Fungi

AM Fungi *Glomus geosporum* inoculum containing about 60 – 65 spores per 5g was procured from International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

Table 1. Experimental set up

Treatments	Meaning
S- M-	- Salinity, - Mycorrhiza
S+ M-	+ Salinity, - Mycorrhiza
S+ M+	+ Salinity, + Mycorrhiza
S- M+	- Salinity, + Mycorrhiza

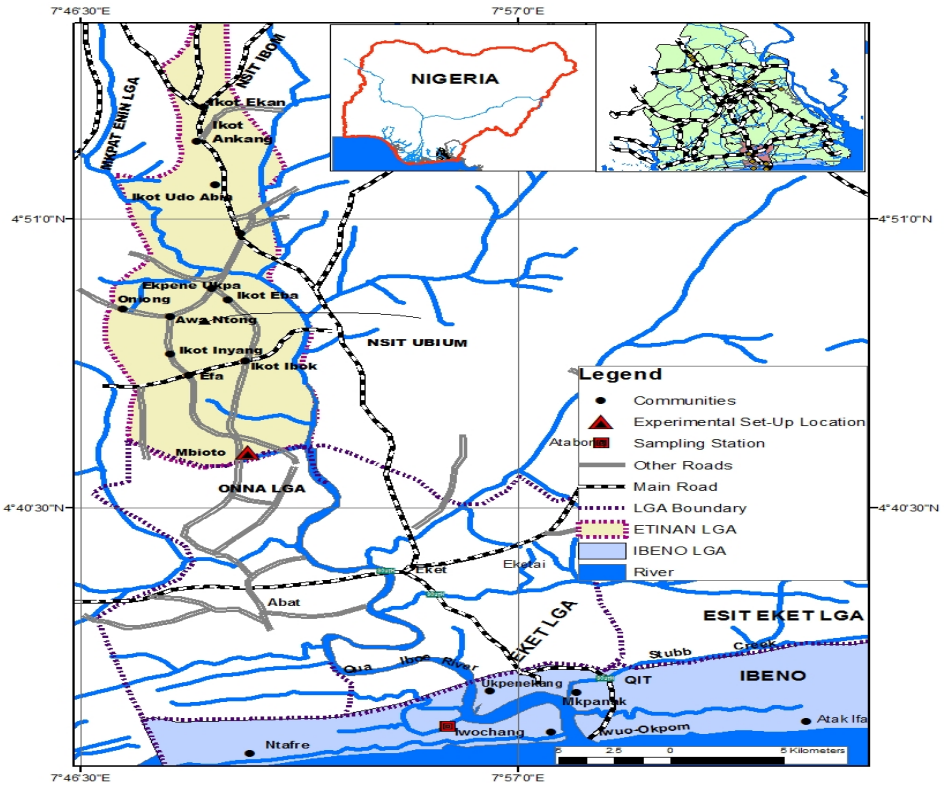

Figure 1. Map showing saline water/soil collection and experimental set-up locations (Source: Field Data)

Soil Analysis

The soil samples were taken and air-dried at room temperature and ground in a wooden mortar to pass through a 2 mm mesh sieve and stored in labelled bags. Sub-samples were taken from each soil sample and analyzed for physico-chemical properties of the soil. All soil analyses were carried out in the Soil Science Department of the University of Uyo, Uyo, Akwa Ibom State. Soil samples were analyzed following the standard procedures outlined by the Association of Official Analytical Chemist [17] procedure for wet acid digestions.

Photosynthetic Pigments Estimation

The atLeaf chlorophyll meter was used for non-destructive estimation of the total chlorophyll and separated into chlorophyll a, b and carotenoids contents of *T. occidentalis.* The atLeaf chlorophyll meter was pinned on the leaf surface and the readings were taken in triplicates.

Mineral Content Analysis

Standard methods of AOAC [18] and Khan *et al*. [19] were used to analyse the mineral contents: Macro-Kjeldahl method was used to determine Nitrogen (N), while atomic absorption spectrophotometer (AAS), flame photometry and spectrophotometry was employed to determine magnesium (Mg), potassium (K), calcium (Ca), sodium (Na) and phosphorus (P) of plant samples.

Quantification of Arbuscular Mycorrhizal Colonization in Plant Roots

About 2 – 4 cm of *T. occidentalis* feeder roots were separately collected for colonization assessment and fixed in 50% ethanol. Running tap water was used to rinse the fixed roots before properly clearing the roots in 10% KOH w/v and autoclaved for about 15 minutes at 121 ^{0}C. Using a fine sieve, cleared roots were collected and rinsed with tap water a number of times before being conveyed into the staining solution. 5% ink diluted in vinegar (5% acetic acid) was used to stain the feeder roots. The feeder roots segments were drenched in the ink and left in staining solution at room temperature for one day. Destaining was done using 50% glycerol for 1 hour [20].

A 9 cm diameter petri plate with a filter paper marked with grid lines was used to randomly disperse the stained roots. x40 magnification was used to scan through horizontal and vertical gridlines using a dissecting microscope. Mycorrhizal colonization can then be calculated as the proportion of root length that is mycorrhizal infected and total root length can then be said to be a conversion factor derived from the total length of grid lines and the area of the dish. Mycorrhizal root colonization was thus determined by the valuation of percentage of root segments containing hyphae, arbuscules and vesicles [21].

$$\mathrm{MC} = \frac{\text{Total number of roots infected intersecting gridlines}}{\text{Total number of roots intersecting gridlines}} \times 100$$

Determination of Mycorrhizal Dependency (MD)

Mycorrhizal dependency (MD) was calculated according to the following formula:

$$\mathrm{MD} = \frac{\text{DW inoculated Plants} - \text{DW non-inoculated Plants}}{\text{DW inoculated Plants}} \times 100$$

Statistical Analysis

Complete randomized design was employed in this study with three (3) replicates for each plant. Analysis of variance (ANOVA) was used to assess all data obtained in this study using the Statistical package for Social Sciences (SPSS) and data are presented as standard error of mean (± S.E.M.) of triplicate experiments. Duncan's multiple range tests was used to separate and compare differences between the means. However, a probability level of p=0.05 was considered statistically significant.

Results

Physicochemical Properties of the Experimental Soils (Saline and Non-saline Soil)

The t-test analysis carried out on the physicochemical properties of the experimental soils (saline and garden soils) indicated significant (p=0.05) differences between the two soil types in; pH, total nitrogen, available phosphorus, silt, clay, sand, Ex. Ca, Ex. Mg, Ex. K, OC, Ex. Na and EC. However, there was no significant (p=0.05) difference in Ex. Acidity, ECEC and base saturation (Table 2).

Table 2. Physicochemical properties of the experimental soils

S/No.	Parameters	Garden Soil	Saline Soil	t-values
1.	pH	6.78	7.75	-56.655*
2.	Total Nitrogen (%)	2.27	0.49	6.928*
3.	Available P. (mg/kg)	36.31	24.66	663.929*
4.	Silt (%)	4.00	5.60	-51.995*
5.	Clay (%)	4.20	12.00	-193.742*
6.	Sand (%)	92.04	82.40	261.909*
7.	Ex. Ca (cmol./kg)	5.25	2.97	-148.956*
8.	Ex. Mg (cmol./kg)	4.36	3.80	23.714*
9.	Ex. Na. (cmol./kg)	0.41	8.81	1.000*
10.	Ex. K. (cmol./kg)	6.98	1.48	43.301*
11.	Organic Carbon (%)	5.61	1.61	-599.00*
12.	Exchangeable acidity (meq/100g)	3.56	3.20	20.785
13.	ECEC (cmol./kg)	20.56	20.26	-228.669
14.	Base saturation (%)	82.68	84.20	-64.341
15	EC. (dS/m)	0.32	7.80	-93.260*

* Significant at t = 0.05, Ex – Exchange, ECEC – Effective cation exchange capacity, EC – Electrical conductivity

***G. geosporum* inoculation on the Photosynthetic Pigments of *T. occidentalis* cultivated on Saline Soil**

Photosynthetic pigments (such as chlorophyll a, b and carotenoids) analysis of *T. occidentalis* cultivated in saline soils taken at 9 WAP revealed significantly (p=0.05) reduction in chlorophyll a, b and carotenoids when compared to the control while inoculation with *G. geosporum* showed significantly (p=0.05) increase in chlorophyll a, b and carotenoids of *T. occidentalis.*

Table 3. Effects of AMF inoculation on the photosynthetic pigments content of *T. occidentalis* grown in saline soil

Treatments	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Carotenoids (mg/g)	Total Photosynthetic Pigments (mg/g)
S- M-	*41.43 ± 3.41[a]	12.83 ± 1.67[a]	3.80 ± 0.34[a]	58.06 ± 4.13[a]
S+ M-	29.16 ± 1.24[b]	6.22 ± 0.12[b]	2.68 ± 0.21[b]	38.06 ± 4.05[c]
S+ M+ (*Gg*)	38.43 ± 2.11[a]	9.57 ± 1.33[a]	3.66 ± 0.57[a]	51.66 ± 3.44[b]
S- M+ (*Gg*)	42.02 ± 2.15[a]	13.14 ± 0.96[a]	4.16 ± 2.05[a]	59.32 ± 3.67[a]

*Mean of three replicates ± SEM. [a]Means within of each column followed by different letters are significantly different at p=0.05 according to Duncan's Multiple Range Test. S- (No salinity), M- (No mycorrhiza), S+ (Plus salinity), M+ (Plus mycorrhiza), (Gg) – *Glomus geosporum*.

***G. geosporum* inoculation on the Mineral Nutrient Contents of *T. occidentalis* cultivated on Saline Soil**

N, P, K, Mg and Ca contents of *T. occidentalis* were observed to be significantly (p=0.05) reduced in saline soil treatments when compared to the control while, N showed slight decrease in saline soil treatments, while Na increased in saline soil treatments (Table 4). Inoculation of *T. occidentalis* with *G. geosporum* showed significant (p=0.05) increased in N, P, K, Mg and Ca contents of the test plant in saline and non-saline soil treatments (Table 4).

Table 4. Effects of arbuscular mycorrhizal fungi (AMF) inoculation on the mineral nutrient contents of *T. occidentalis* grown in saline soil

Treatments	N (%)	P (mg/kg)	K (mg/kg)	Mg (mg/kg)	Ca (mg/kg)	Na (mg/kg)
S- M-	*5.84[a]	424.11[b]	3215.00[b]	326.00[b]	1640.00[b]	500.00[c]
S+ M-	3.13[b]	312.31[c]	1220.00[d]	107.04[d]	873.00[d]	2920.13[a]
S+ M+ (*Gg*)	4.07[a]	246.77[d]	1570.00[c]	294.06[c]	1080.00[c]	1011.00[b]
S- M+ (*Gg*)	6.44[a]	503.00[a]	3492.21[a]	410.00[a]	1660.00[a]	422.00[d]

*Mean of three replicates. [a]Means within of each column followed by different letters are significantly different at p=0.05 according to Duncan's Multiple Range Test. S- (No salinity), M- (No mycorrhiza), S+ (Plus salinity), M+ (Plus mycorrhiza), (Gg) – *Glomus geosporum*.

Significant (p=0.05) reduction was observed in the percentage AM fungi root colonization of *T. occidentalis* in saline soil treatments (Table 5). However, inoculated saline soil treatment showed significant (p=0.05) increased in the %AMF root colonization.

Table 5. Arbuscular mycorrhizal fungi (AMF) colonization of *T. occidentalis* grown in saline soil

Non-inoculated treatment	Root colonization (%)	Mycorrhizal Dependency (%)	Inoculated treatments	Root colonization (%)	Mycorrhizal Dependency (%)
S-M-	0.00	0.00	S+M+ (*Gg*)	*22.41[b]	100.00[a]
S+M-	0.00	0.00	S-M+ (*Gg*)	53.97[a]	15.13[b]

*Mean of three replicates. [a]Means within of each column followed by different letters are significantly different at p=0.05 according to Duncan's Multiple Range Test. S- (No salinity), M- (No mycorrhiza), S+ (Plus salinity), M+ (Plus mycorrhiza), (Gg) – *Glomus geosporum*.

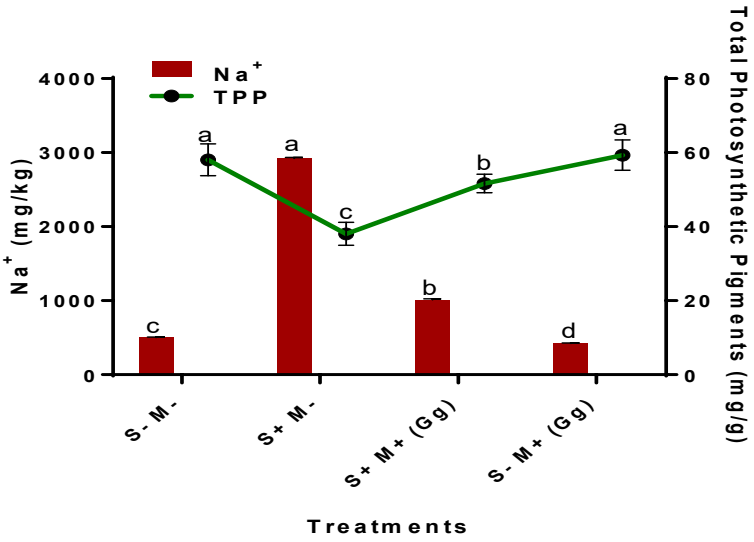

Figure 2. Influence of AMF inoculation on foliar Na^+ uptake in *T. occidentalis* as it affects total photosynthetic pigments

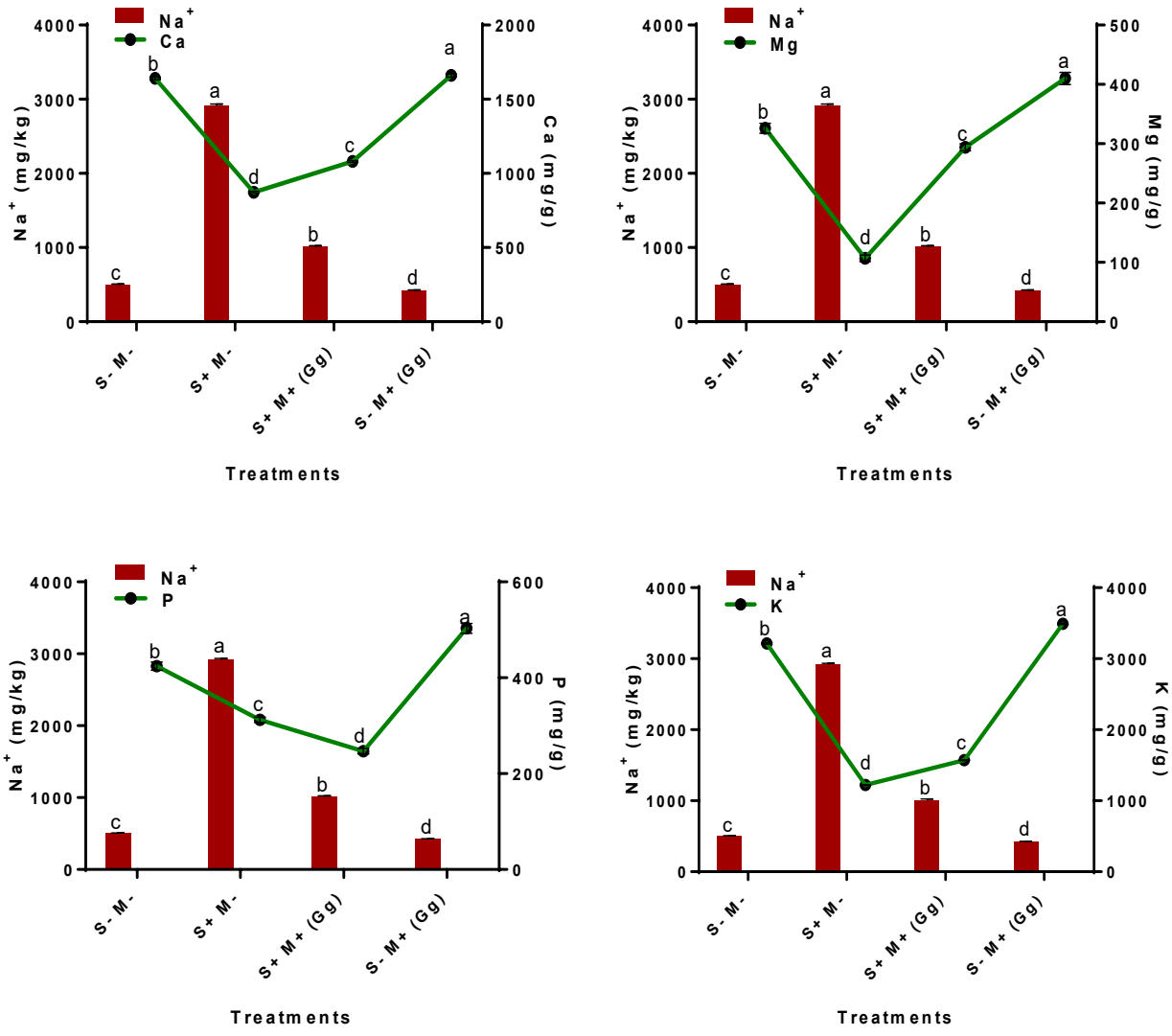

Figure 3. Influence of AMF inoculation on foliar Na^+ uptake in *T. occidentalis* as it affects its K, Ca, P and Mg content

Discussion

Analysis of the saline and garden soils used in this study revealed significant (p=0.05) variations in their soil physico-chemical parameters. Significant (p=0.05) increase in parameters such as pH, EC and Ex Na^+ was observed in the saline soil while there was a decrease in organic carbon, total nitrogen and available phosphorus in saline soil. This observation is in line with the work of Miller and Gardiner [22] who reported an increase in pH and EC in saline soils in New Jersey due to salt stress. Deleke and Akomolafe [23] also made similar findings as they observed an increase in pH, EC and Ex Na^+ in saline soils and a decrease in organic carbon, organic matter, total nitrogen and phosphorus in salinity influenced soils in Nigeria. The reduction in the content of the soil organic carbon content can be attributed to two antagonizing factors: the reduction of plant-soil inputs and the reduction in the rate of decomposition in the soil [24].

Photosynthetic pigments contents results showed that salt stress significantly (p=0.05) reduced the total photosynthetic pigments contents in *T. occidentalis* in both mycorrhizal and non-mycorrhizal saline treated plants. Non-mycorrhizal plants in saline soil treatments were more severely affected than *T. occidentalis* inoculated with *G. geosporum*. This observation is in agreement with the work of Jing *et al*. [25] who reported that the total chlorophyll content significantly decreased in *Suaeda aralocaspica* exposed to high salinity. They attributed the decrease to the destruction of the chloroplast structure. The influence of AMF on photosynthesis has been reported in many mycorrhizal plants growing under salinity stress [26, 27, 28, 29]. Under saline soil conditions, increase in the photosynthetic rate in AM fungi-colonized plants can be attributed to the lowered intercellular CO_2 concentration in mycorrhizal plants, as a higher photosynthetic capability results in upsurges in water use efficiency for the incorporation of extra carbon per unit water transpiration [28].

Significant (p=0.05) reduction in N, P, K, Mg and Ca content of *T. occidentalis* was observed in saline soil treatments according to this study, while foliar uptake and accumulation of Na^+ was significantly (p=0.05) increased in saline soil treatments than in non-saline treatments. Comparing the influence of Na^+ foliar uptake on other minerals, it was observed that Na^+ accumulation had negative effects on the mineral composition of *T. occidentalis*. This observation corroborates the work of Robert *et al*. [30] who reported that injury in crops results from exposure to NaCl which lowers soil water potential thereby resulting in osmotic stress. Ullah *et al.* [31] in their study on tomato plants irrigated with sea water showed excessive take up of Na+ and Cl- and a remarked decrease in P and Fe uptake. The uptake of important mineral elements in the soils such as P, K, Ca^{+2}, $^+$, Mg^{+2} and N is antagonized by excessive presence of Na^+ and Cl^- in soil solution, as a result of this antagonistic relationship; proper acquisition of plant mineral nutrients is hampered thus limiting plant growth and biomass yield. In this study, it was also observed that the minerals composition of *T. occidentalis* under saline and non-saline treatments was significantly increased with AMF *G. geosporum* inoculation. A higher N, P, K, Mg and Ca concentration in mycorrhizal than non-mycorrhizal plants can favourably assuage the toxic effects of NaCl by inducing a higher K^+/Na^+ rate leading to salt adaptation [32]. Cantrell and Linderman [33] reported increased N, K, Mg, P and Ca uptake in mycorrhizal lettuce.

The reduction in AM fungi root colonization on the test plant observed in this study is in agreement with earlier studies reporting that addition of salt to soil inhibits hyphal growth, which subsequently reduces the spread of mycorrhizal colonization [34, 35]. It was also observed in this study that mycorrhizal dependency (MD) varied with saline treatments. Root colonization of *T. occidentalis* by *G. geosporum* showed great dependency of these plants on the AMF when compared to the purely saline non-mycorrhizal treatment. This corroborates the work of Beltrano *et al*. [36] who demonstrated the favourable association amongst pepper and *G. intaradices* (currently known as *R. irregularis*), however, soil salinity decreased AM fungi root colonization. Also, pepper plants recorded a very high dependency on the AM fungi [36].

Conclusion

Abiotic stress such as soil salinity is a major global limitation to plant growth, production and yield as also observed in this research. Observations recorded in this study showed how soil salinity deleteriously affected physicochemical properties of the saline soil when compared to the garden soil, thus resulting in negative effects on photosynthetic pigments, N, P, K, Mg and Ca concentration, AM fungi root colonization and dependency of *T. occidentalis*. AM fungi (*G. geosporum*) symbiotic relationship with *T. occidentalis* produced enhanced improvements on the physiology of the test plant. Using different mechanisms *T. occidentalis* by association with *G. geosporum* showed better salt tolerance thank the uninoculated plants. *G. geosporum* was able to impose some physiological and root morphological changes such as an extensive network of the mycorrhizal-plant roots to improve water and mineral nutrient uptake. Physiologically *G. geosporum* inoculation enriched *T. occidentalis* vigour, attuned the rate of K^+/Na^+ which restored nutrient and water balance in the plant and directly resulting in the enhancement of salt tolerance in *T. occidentalis* seedlings, thus improving growth and yield.

Conflict of Interest

The authors declare that there is no conflict of interest between them.

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