



## Na<sup>+</sup> exclusion and na<sup>+</sup>/k<sup>+</sup> ratio adjustment by mycorrhiza enhances macro/micro nutrients uptake in two members of *Cucurbitaceae* family under salt stress

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### Abstract

This research investigated the influence of inoculating *Telfairia occidentalis* and *Cucurbita maxima* with arbuscular mycorrhizas (*Rhizophagus irregularis* and *Glomus geosporum*) with poultry manure in Na<sup>+</sup>/K<sup>+</sup> ratio adjustment and plant mineral nutrition. Saline soil used had an electrical conductivity (EC) of 6.70 dS/m, while the garden soil had an EC of 0.29 dS/m. Mineral analysis of *C. maxima* and *T. occidentalis* leaves revealed increased uptake and accumulation of Na<sup>+</sup>, Cl<sup>-</sup>, Zn and a high Na<sup>+</sup>/K<sup>+</sup> ratio, while N, P, K, Mg, Ca, Fe and Mn were significantly (p=0.05) reduced in saline soil treatments. Inoculation with mycorrhiza alone or together with PM increased the minerals in the two test plants both in saline and non-saline treatments. The mycorrhizas aided the maintenance of favorable K<sup>+</sup>/Na<sup>+</sup> ratio equivocating the disruption of K<sup>+</sup> homeostasis. The capacity of plants to maintain a high cytosolic K<sup>+</sup>/Na<sup>+</sup> ratio is one of the key determinants of plant salt tolerance.

**Keywords:** *Cucurbita maxima*, *Cucurbitaceae*, *Glomus geosporum*, *Rhizophagus irregularis*, salinity, *Telfairia occidentalis*

### 1. Introduction

Soil solution contains various important soluble salts and is considered as one of the best media for plant growth and development [1]. Plants through their roots absorb these soluble salts and translocate them to different parts where these salts are required to perform numerous metabolic activities. However, excessive salts in soil reduce plant water and nutrient uptake and disrupt the distribution of ions at both the cellular and the whole-plant levels, thereby inducing osmotic and ionic imbalances [1]. Such drastic changes result in stunted plant growth and development and can lead to death of the plant. Salinization of soils is one of the most predominant agricultural problems commonly occurring in the arid, semiarid and low-lying coastal areas of the world [2, 3]. According to the Food and Agricultural Organization (FAO), about 7% of the global soil surface is affected by salt, out of which 15.7 Mha is in North America, 1.9 Mha in Mexico and Central America, 129.16 Mha in South America, 80.43 Mha in Africa, 85.10 Mha in South and West Asia, 19.98 Mha in Southeast Asia, 211.68 Mha in North and Central Asia, 357.33 Mha in Australia, and 52.08 Mha in Europe [4, 5].

Higher accumulation of salts like Na<sup>+</sup> and Cl<sup>-</sup> in plant tissues leads to oxidative damage (also considered as secondary stress), affecting integrity of plant membranes (damage to lipids, proteins, and nucleic acids), impairing activities of biocatalysts and functioning of photosynthetic apparatus. This is ascribed to the deleterious effects of reactive oxygen species (ROS) which are often generated by salt stress [1, 6]. Globally, salinization of soil is increasing due to rise in the sea levels by climate change and also due to wrong irrigation practices of agricultural lands [7, 8]. In some areas, it is increasing due to extensive use of salt on roads to prevent frozen glaze in winter [7].

Some microorganisms, particularly beneficial bacteria and fungi can improve plant performance under stress

environments and consequently enhance yield [9]. Microorganisms such as arbuscular mycorrhizal (AM) fungi are able to colonize plants, in their natural environment. AM fungi are associated with the roots of over 80 %terrestrial plant species [10]. AM fungi have been shown to promote plant growth and salinity tolerance by many researchers. They promote salinity tolerance by utilizing various mechanisms, such as: Enhancing nutrient uptake [11], Producing plant growth hormones [12], Improving rhizospheric and soil conditions [13], Improvement in photosynthetic activity or water use efficiency [14], Accumulation of compatible solutes [12] and Production of higher antioxidant enzymes [1].

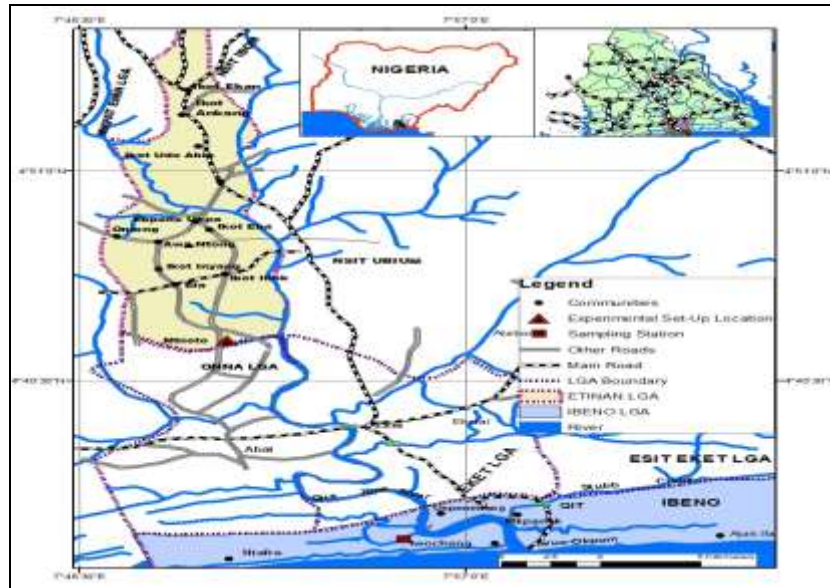
Thus, this research was undertaken to investigate the influence of inoculating two (2) different vegetables (*Telfairia occidentalis* Hook F. and *Cucurbita maxima* Duch.) belonging to the family *Cucurbitaceae* with arbuscular mycorrhiza (*Rhizophagus irregularis* and *Glomus geosporum*) with poultry manure in K<sup>+</sup>/Na<sup>+</sup> ratio adjustment and plant mineral nutrition.

### 2. Materials and Methods

#### 2.1 Study Area

Saline soil and salt water were collected from the saline ecosystem of Iwuochang, Ibeno Local Government Area (Latitude 4.56°N and Longitude 7.57°E), Akwa Ibom State, Nigeria, with an annual rainfall of about 4021 mm and mean temperature variation of 22 – 31°C. The experiment was set up in a safe and secured environment at Mbioto 1, Etinan Local Government Area (Latitude 4.51°N and Longitude 7.50°E), Akwa Ibom State, Nigeria, with an annual rainfall of about 4000 mm and mean temperature variation of 26 – 36°C [15]. 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.



**Fig 1:** Map showing saline water/soil collection and experimental set-up locations.

**2.2 Propagation and Mycorrhizal Inoculation**

Arbuscular Mycorrhizal fungi *R. irregularis* and *G. geosporum* were multiplied on maize grown in the greenhouse of the Department of Botany for 16 weeks. The colonized maize roots were used as an inoculum (25 g fresh weight per pot containing approximately 130 spores) was placed in the pot at 15 cm depth, before planting.

**2.3 Experimental Design**

This experiment was set up in a complete block design with all treatments replicated thrice for both *C. maxima* and *T. occidentalis*. This gave a total of twelve (12) treatments for each plant with three (3) replicates totaling seventy-two (72) combinations for each plant making it 144 combinations for *C. maxima* and *T. occidentalis* (Table 1).

**Table 1:** Experimental design

Treatments	Meaning
S- M- P-	- Salinity, - Mycorrhiza, - Poultry
S+ M- P-	+ Salinity, - Mycorrhiza, - Poultry
S+ M+ P- ( <i>Gg</i> )	+ Salinity, + Mycorrhiza ( <i>G. geosporum</i> ), - Poultry
S+ M+ P- ( <i>Ri</i> )	+ Salinity, + Mycorrhiza ( <i>R. irregularis</i> ), - Poultry
S+ M- P+	+ Salinity, - Mycorrhiza, + Poultry
S+ M+ P+ ( <i>Gg</i> )	+ Salinity, + Mycorrhiza ( <i>G. geosporum</i> ), + Poultry
S+ M+ P+ ( <i>Ri</i> )	+ Salinity, + Mycorrhiza ( <i>R. irregularis</i> ), + Poultry
S- M+ P- ( <i>Gg</i> )	- Salinity, + Mycorrhiza ( <i>G. geosporum</i> ), - Poultry
S- M+ P- ( <i>Ri</i> )	- Salinity, + Mycorrhiza ( <i>R. irregularis</i> ), - Poultry
S- M+ P+ ( <i>Gg</i> )	- Salinity, + Mycorrhiza ( <i>G. geosporum</i> ), + Poultry
S- M+ P+ ( <i>Ri</i> )	- Salinity, + Mycorrhiza ( <i>R. irregularis</i> ), + Poultry
S- M- P+	- Salinity, - Mycorrhiza, + Poultry

**2.4 Planting**

Five (5) seeds each of *C. maxima* and *T. occidentalis* were sown in their respective plastic pots filled with about 10 kg of sterilized soils. Arbuscular mycorrhiza fungi was inoculated by placing 25g of soil/root fragments containing 60 – 65 spores per 5g in planting hole at 15 cm depth, before planting the *C. maxima* and *T. occidentalis*. Following seedling emergence, the plants inoculated were allowed to establish for up to 2 weeks before being treated with the first dose of saline water. This was to ensure the establishment of AM colonization and avoid sudden plant death due to salinity shock. The dose of saline water used was 100 ml per plant pot every 3 days.

**2.5 Water Analysis and Physico-chemical Properties of Experimental Soils**

Water and soil samples were analyzed following the

Standard procedures outlined by the Association of Official Analytical Chemist <sup>[16]</sup> procedure for wet acid digestions.

**2.6 Determination of Mineral Content**

The plant samples were sent to Ministry of Science and Technology, Akwa Ibom State for mineral analysis. Mineral contents: Nitrogen (N) was determined using the Macro-Kjeldahl method while calcium (Ca), magnesium (Mg), potassium (K), iron (Fe), manganese (Mn), Chlorine (Cl), zinc (Zn), sodium (Na) and phosphorus (P) of plant samples were determined by atomic absorption spectrophotometer (AAS), flame photometry and spectrophotometry according to the methods of AOAC <sup>[17, 18]</sup>.

**2.7 Statistical Analysis**

All data in the present study were subjected to analysis of

variance (ANOVA) using Statistical package for Social Sciences and data are presented as standard error of mean ( $\pm$  S.E.M.) of triplicate experiments. The student's t-test was used to determine the significant difference between means of the soil and water parameters analyzed using Statistical package for social science (SPSS). The differences between the means were separated and compared using the Duncan's multiple range tests. However, a probability level of  $p=0.05$  was considered statistically significant.

### 3. Results and Discussions

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

(Table 2). Similarly, the t-test analysis carried out on the properties of the experimental irrigation water (saline and freshwater) indicated significant ( $p=0.05$ ) difference between the two water types in; pH, EC, TDS, alkalinity and salinity (Table 2).

This observation is in line with the work of Miller and Gardiner [19] who reported an increase in pH and EC in saline soils in New Jersey due to salt stress. Deleke and Akomolafe [20] also made similar findings as they observed an increase in pH, EC and Ex  $\text{Na}^+$  in saline soils and a decrease in organic carbon, organic matter, total nitrogen and phosphorus in salinity influenced soils in Nigeria. Soil organic carbon content is influenced by two opposing factors: reduced plant inputs and reduced rates of decomposition [21].

**Table 2:** Physicochemical properties of the experimental soils

S/No.	Parameters	Garden Soil	Saline Soil	t-values
1.	pH	6.69	7.40	-4.786*
2.	Total Nitrogen (%)	2.36	0.36	2.982*
3.	Available P. (mg/kg)	32.11	21.78	24.750*
4.	Silt (%)	4.55	5.84	-3.400
5.	Clay (%)	3.48	10.22	-15.437*
6.	Sand (%)	91.97	83.94	13.890*
7.	Ex. Ca (cmol./kg)	4.11	3.54	1.121*
8.	Ex. Mg (cmol./kg)	5.61	3.84	4.773*
9.	Ex. Na. (cmol./kg)	0.28	6.48	7.257*
10.	Ex. K. (cmol./kg)	4.05	0.43	70.679*
11.	Organic Carbon (%)	6.48	1.73	-22.922*
12.	EC. (dS/m)	0.29	6.70	-15.322*
Water Parameters				
1.	pH	7.70	6.70	3.273
2.	EC ( $\mu\text{S}/\text{cm}$ )	3080.00	27.70	13.063*
3.	TDS	1021.00	11.00	7.063*
4.	Acidity (mg/l as $\text{CaCO}_3$ )	80.00	95.40	-1.130
5.	Alkalinity (mg/l as $\text{CaCO}_3$ )	138.00	53.20	76.468*
6.	Salinity (ppt)	33.21	0.32	20.839*

\* Significant at  $t = 0.05$ , Ex – Exchange, EC – Electrical conductivity

Mineral nutrient contents of *C. maxima* and *T. occidentalis* such as; P, K, Mg and Ca were significantly ( $p=0.05$ ) reduced in saline soil treatments when compared to the control. N, Mn and Zn showed slight and irregular decrease in saline soil treatments, while Na, Cl and  $\text{Na}^+/\text{K}^+$  ratio calculated increased in saline soil treatments (Table 3 and 4). Amelioration of the saline soil with poultry manure alone also enhanced the mineral nutrient contents of *C.*

*maxima* and *T. occidentalis*. Inoculation with AMF alone or together with poultry manure amelioration significantly ( $p=0.05$ ) increased the mineral nutrient contents in the two test plants both in saline and non-saline soil treatments also adjusting the  $\text{Na}^+/\text{K}^+$  ratio hence leading to reduced  $\text{Na}^+$  uptake resulting in the uptake of essential nutrients (Table 3 and 4).

**Table 3:** Effects of arbuscular mycorrhizal fungi (AMF) inoculation on the mineral nutrient contents and  $\text{Na}^+/\text{K}^+$  ratio of *C. maxima* grown in saline soil ameliorated with poultry manure

Treatments	N (%)	P (mg/kg)	K (mg/kg)	Mg (mg/kg)	Ca (mg/kg)	Na (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Cl (mg/kg)	Zn (mg/kg)	$\text{Na}^+/\text{K}^+$ ratio
S- M- P-	*4.88 <sup>a</sup>	860.40 <sup>c</sup>	4430.00 <sup>e</sup>	558.99 <sup>c</sup>	2810.00 <sup>b</sup>	510.22 <sup>d</sup>	18.77 <sup>a</sup>	68.50 <sup>a</sup>	545.00 <sup>d</sup>	102.11 <sup>c</sup>	0.12 <sup>c</sup>
S+ M- P-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S+ M+ P- (Gg)	3.48 <sup>a</sup>	410.00 <sup>f</sup>	2010.03 <sup>f</sup>	330.20 <sup>e</sup>	1210.00 <sup>i</sup>	1407.00 <sup>c</sup>	16.40 <sup>a</sup>	62.70 <sup>a</sup>	1034.00 <sup>c</sup>	112.00 <sup>a</sup>	0.70 <sup>b</sup>
S+ M+ P- (Ri)	3.13 <sup>a</sup>	504.02 <sup>f</sup>	2009.00 <sup>f</sup>	407.00 <sup>d</sup>	1078.10 <sup>j</sup>	1520.10 <sup>b</sup>	12.00 <sup>a</sup>	63.80 <sup>a</sup>	1602.10 <sup>b</sup>	104.04 <sup>b</sup>	0.76 <sup>b</sup>
S+ M- P+	2.48 <sup>a</sup>	361.21 <sup>b</sup>	1803.10 <sup>g</sup>	304.70 <sup>f</sup>	880.05 <sup>d</sup>	2044.02 <sup>a</sup>	11.70 <sup>b</sup>	58.20 <sup>b</sup>	2105.00 <sup>a</sup>	74.50 <sup>c</sup>	1.13 <sup>a</sup>
S+ M+ P+ (Gg)	4.91 <sup>a</sup>	878.00 <sup>b</sup>	4610.10 <sup>c</sup>	580.02 <sup>b</sup>	3001.00 <sup>f</sup>	502.12 <sup>d</sup>	17.22 <sup>b</sup>	67.99 <sup>a</sup>	470.00 <sup>f</sup>	104.00 <sup>b</sup>	0.11 <sup>c</sup>
S+ M+ P+ (Ri)	4.87 <sup>a</sup>	807.01 <sup>e</sup>	4451.11 <sup>d</sup>	562.77 <sup>c</sup>	2870.01 <sup>g</sup>	511.04 <sup>d</sup>	16.84 <sup>a</sup>	66.70 <sup>a</sup>	512.00 <sup>e</sup>	107.80 <sup>b</sup>	0.11 <sup>c</sup>
S- M+ P- (Gg)	5.02 <sup>a</sup>	878.23 <sup>b</sup>	4613.00 <sup>b</sup>	585.00 <sup>b</sup>	3048.00 <sup>d</sup>	446.70 <sup>f</sup>	18.69 <sup>a</sup>	68.78 <sup>a</sup>	452.10 <sup>g</sup>	110.01 <sup>b</sup>	0.10 <sup>c</sup>
S- M+ P- (Ri)	4.99 <sup>a</sup>	820.20 <sup>d</sup>	4613.22 <sup>b</sup>	598.70 <sup>a</sup>	3070.00 <sup>c</sup>	462.00 <sup>e</sup>	18.02 <sup>a</sup>	68.99 <sup>a</sup>	455.02 <sup>g</sup>	108.10 <sup>b</sup>	0.10 <sup>c</sup>
S- M+ P+ (Gg)	5.12 <sup>a</sup>	892.00 <sup>a</sup>	4628.10 <sup>a</sup>	591.00 <sup>a</sup>	3120.04 <sup>b</sup>	412.12 <sup>g</sup>	19.01 <sup>a</sup>	72.45 <sup>a</sup>	425.00 <sup>h</sup>	110.01 <sup>b</sup>	0.09 <sup>c</sup>

S- M+ P+ (Ri)	5.47 <sup>a</sup>	896.22 <sup>a</sup>	4630.00 <sup>a</sup>	592.10 <sup>a</sup>	3151.00 <sup>a</sup>	409.80 <sup>h</sup>	19.22 <sup>a</sup>	72.55 <sup>a</sup>	410.00 <sup>i</sup>	109.02 <sup>b</sup>	0.09 <sup>c</sup>
S- M- P+	4.92 <sup>a</sup>	868.00 <sup>c</sup>	4612.00 <sup>c</sup>	584.12 <sup>b</sup>	3012.00 <sup>c</sup>	503.00 <sup>d</sup>	17.67 <sup>a</sup>	67.54 <sup>a</sup>	462.21 <sup>f</sup>	102.70 <sup>b</sup>	0.11 <sup>c</sup>

\*Mean of three replicates. <sup>a</sup>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), P- (No poultry manure), S+ (Plus salinity), M+ (Plus mycorrhiza), P+ (Plus poultry droppings), (Gg) – *Glomus geosporum*, (Ri) – *Rhizophagus irregularis*, - (means the plants were dead).

**Table 4:** Effects of arbuscular mycorrhizal fungi (AMF) inoculation on the mineral nutrient contents and Na<sup>+</sup>/K<sup>+</sup> ratio of *T. occidentalis* grown in saline soil ameliorated with poultry manure

Treatments	N (%)	P (mg/kg)	K (mg/kg)	Mg (mg/kg)	Ca (mg/kg)	Na (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Cl (mg/kg)	Zn (mg/kg)	Na <sup>+</sup> /K <sup>+</sup> ratio
S- M- P-	*5.84 <sup>a</sup>	424.11 <sup>d</sup>	3215.00 <sup>d</sup>	326.00 <sup>c</sup>	1640.00 <sup>d</sup>	500.00 <sup>a</sup>	31.09 <sup>a</sup>	102.12 <sup>a</sup>	625.00 <sup>f</sup>	37.41 <sup>a</sup>	0.16 <sup>f</sup>
S+ M- P-	3.13 <sup>b</sup>	312.31 <sup>f</sup>	1220.00 <sup>i</sup>	107.04 <sup>g</sup>	873.00 <sup>h</sup>	2920.13 <sup>b</sup>	26.41 <sup>a</sup>	87.01 <sup>b</sup>	3140.00 <sup>a</sup>	32.11 <sup>a</sup>	2.39 <sup>a</sup>
S+ M+ P- (Gg)	4.07 <sup>a</sup>	246.77 <sup>i</sup>	1570.00 <sup>g</sup>	294.06 <sup>e</sup>	1080.00 <sup>c</sup>	1011.00 <sup>c</sup>	26.00 <sup>a</sup>	88.42 <sup>b</sup>	1150.00 <sup>c</sup>	34.05 <sup>a</sup>	0.64 <sup>c</sup>
S+ M+ P- (Ri)	4.62 <sup>a</sup>	284.60 <sup>h</sup>	1710.00 <sup>f</sup>	300.00 <sup>d</sup>	1120.0 <sup>f</sup>	846.13 <sup>d</sup>	28.71 <sup>a</sup>	86.70 <sup>b</sup>	900.02 <sup>d</sup>	34.00 <sup>a</sup>	0.49 <sup>d</sup>
S+ M- P+	3.64 <sup>b</sup>	242.00 <sup>i</sup>	1402.04 <sup>h</sup>	263.05 <sup>f</sup>	1012.01 <sup>g</sup>	1201.00 <sup>e</sup>	18.77 <sup>b</sup>	65.43 <sup>c</sup>	1200.00 <sup>b</sup>	34.71 <sup>a</sup>	0.86 <sup>b</sup>
S+ M+ P+ (Gg)	5.44 <sup>a</sup>	296.21 <sup>g</sup>	2670.00 <sup>e</sup>	302.00 <sup>d</sup>	1180.00 <sup>e</sup>	620.41 <sup>f</sup>	30.00 <sup>a</sup>	90.22 <sup>b</sup>	721.07 <sup>e</sup>	37.00 <sup>a</sup>	0.23 <sup>e</sup>
S+ M+ P+ (Ri)	5.92 <sup>a</sup>	426.35 <sup>d</sup>	3300.00 <sup>d</sup>	330.04 <sup>c</sup>	1642.11 <sup>d</sup>	470.00 <sup>g</sup>	33.61 <sup>a</sup>	103.00 <sup>a</sup>	601.00 <sup>g</sup>	36.41 <sup>a</sup>	0.14 <sup>f</sup>
S- M+ P- (Gg)	6.07 <sup>a</sup>	463.00 <sup>c</sup>	3470.12 <sup>c</sup>	345.00 <sup>b</sup>	1658.12 <sup>c</sup>	441.11 <sup>h</sup>	33.82 <sup>a</sup>	103.09 <sup>a</sup>	504.09 <sup>i</sup>	37.58 <sup>a</sup>	0.13 <sup>f</sup>
S- M+ P- (Ri)	6.21 <sup>a</sup>	510.01 <sup>b</sup>	3471.00 <sup>c</sup>	325.25 <sup>c</sup>	1655.06 <sup>c</sup>	420.01 <sup>i</sup>	33.22 <sup>a</sup>	104.00 <sup>a</sup>	510.01 <sup>i</sup>	37.00 <sup>a</sup>	0.12 <sup>f</sup>
S- M+ P+ (Gg)	6.44 <sup>a</sup>	503.00 <sup>b</sup>	3492.21 <sup>b</sup>	410.00 <sup>a</sup>	1660.00 <sup>c</sup>	422.00 <sup>i</sup>	34.40 <sup>a</sup>	104.11 <sup>a</sup>	486.00 <sup>j</sup>	37.46 <sup>a</sup>	0.12 <sup>f</sup>
S- M+ P+ (Ri)	6.81 <sup>a</sup>	567.20 <sup>a</sup>	3610.00 <sup>a</sup>	339.00 <sup>b</sup>	1694.00 <sup>a</sup>	410.00 <sup>j</sup>	31.00 <sup>a</sup>	106.00 <sup>a</sup>	462.11 <sup>k</sup>	38.00 <sup>a</sup>	0.11 <sup>f</sup>
S- M- P+	5.99 <sup>a</sup>	331.02 <sup>e</sup>	3214.00 <sup>d</sup>	336.10 <sup>c</sup>	1670.00 <sup>b</sup>	459.22 <sup>k</sup>	33.00 <sup>a</sup>	104.10 <sup>a</sup>	582.00 <sup>h</sup>	37.82 <sup>a</sup>	0.14 <sup>f</sup>

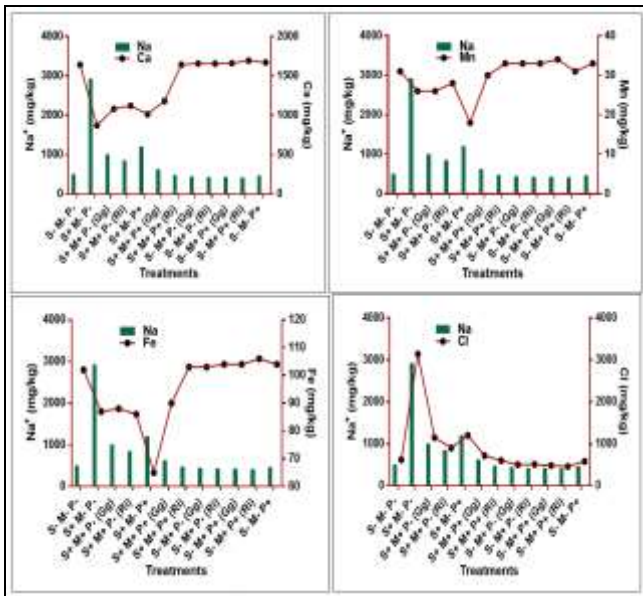
\*Mean of three replicates. <sup>a</sup>Means within of each column followed by different letters are significantly different at p=0.05 according to Duncan's Multiple Range Test. S- (No salinity), M- (No mycorrhiza), P- (No poultry manure), S+ (Plus salinity), M+ (Plus mycorrhiza), P+ (Plus poultry droppings), (Gg) – *Glomus geosporum*, (Ri) – *Rhizophagus irregularis*, - (means the plants were dead).

The mineral composition of *C. maxima* and *T. occidentalis* (N, P, K, Mg, Ca, Fe and Mn) were significantly (p=0.05) reduced in saline soil treatments in this study, while foliar uptake and accumulation of Na<sup>+</sup>, Cl<sup>-</sup> and Na<sup>+</sup>/K<sup>+</sup> ratio were significantly (p=0.05) increased in saline soil treatments than in non-saline treatments. Comparing the influence of Na<sup>+</sup> foliar uptake on other minerals, it was observed that Na<sup>+</sup> accumulation had negative effects on the mineral composition of *C. maxima* and *T. occidentalis*. This observation agrees with the work of Robert *et al.* [22] who reported that exposure to NaCl injures plants in part through lowered soil water potential and the ensuing osmotic stress, but ultimately it may be more injurious via direct toxicity of Na<sup>+</sup> ions. Ullah *et al.* [23] reported that irrigation of tomato plants with seawater increased the uptake of sodium chloride and decreased the uptake of P and Fe. Increased concentration of Na<sup>+</sup> and Cl<sup>-</sup> in soil solution competes with the uptake of vital ions such as Ca<sup>2+</sup>, P, K<sup>+</sup>, Mg<sup>2+</sup> and N; and alters the ideal salt ratios in the soil solution, therefore affecting plant nutrient acquisition and restricting plant growth and biomass.

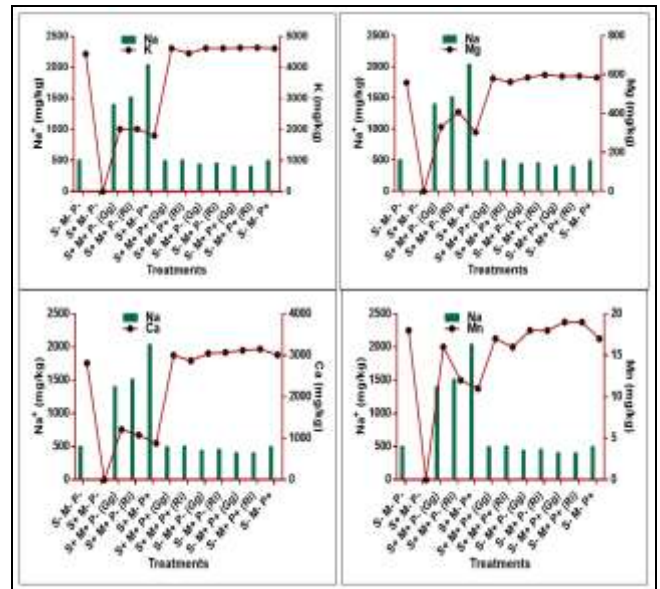
Evelin *et al.* [24] reported that total N concentration in shoot and root of fenugreek plants was severely affected by NaCl-induced salinity in the soil. This negative effect of salt on N uptake of *C. maxima* and *T. occidentalis* is clearly seen in this study. The findings in this study are in agreement with previous reports of Cantrell and Linderman [25] on lettuce and onions plants, Silveira *et al.* [26] on cowpea plants and Colla *et al.* [27] in zucchini plants who all reported reduction of N composition with increase in salinity. Evelin *et al.* [9, 24] reported reduction in the uptake and concentration of P in plant tissues of fenugreek plants under NaCl-induced salinity in the soil. Phosphorus is an essential macronutrient and forms an integral component of several key plant structures in plant cells, including the sugar-phosphate intermediates of respiration and photosynthesis, and the phospholipids that make up the plant membranes. Under salinity stress, the uptake and concentration of P in plant

tissues decreases resulting in reduced and stunted growth, dark green coloration of the leaves, production of slender stems, and senescence of older leaves [11, 28]. These results are also in agreement with the findings of other earlier researchers who reported negative impact of salt stress on Ca<sup>2+</sup> and Mg content of *Acacia nilotica* [29], on *Z. mays* [30], *S. lycopersicum* [31] and fenugreek plants [24]. AMF application improved N uptake of *C. maxima* and *T. occidentalis* and this agrees with the findings of Evelin *et al.* [24] that inoculation with *G. intaradices* (*R. irregularis*) improved the total N concentration in shoot and root of fenugreek plants over non-inoculated plants. Application of AM fungi can result in a more efficient assimilation of N in the host plants due to nitrate assimilation in the extra radical mycelia through the activity of nitrate reductase located in the arbuscular containing cells leading to the formation of arginine and increased production of enzymes controlling the primary nitrogen fixation.

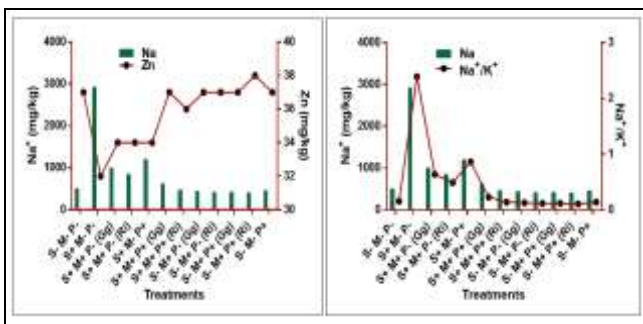
The micronutrients such as Fe and Mn contents of *C. maxima* and *T. occidentalis* were significantly (p=0.05) reduced in saline soil treatments compared to non-saline soil treatments. However, Zn content of *C. maxima* and *T. occidentalis* was slightly increased in saline soil treatments. This possibly could have been as a result of high soil content of Zn in the study soil. In most of the salinity-plant mineral nutrition studies, the micronutrients have drawn the least attention. The availability of most micronutrients depends on pH and EC of the soil solution, as well as the nature of binding sites on organic and inorganic particle surfaces [9]. The mobility of Cu, Fe, Mn and Zn<sup>2+</sup> in the soil solution is low [9]. In saline soils, the solubility of these micronutrients also decreases [9]. These are poorly mobile micronutrients, the effectivity of mycorrhizal plants depends upon the spread of extraradical hyphae in soil, and the reduction of Mn, Cu, Zn<sup>2+</sup> and Fe in mycorrhizal plants with saline soil treatment could have possibly been due to direct effects of salinity on hyphal growth and spread [9].



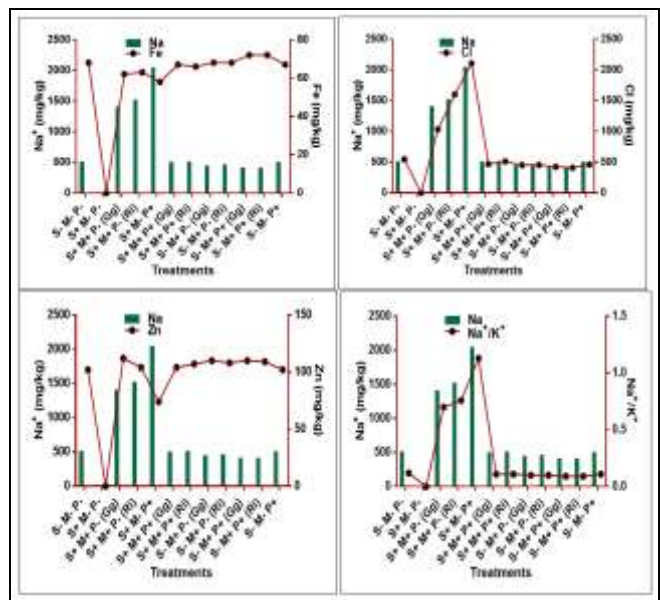
**Fig 2:** Comparative assessment of the influence of foliar Na<sup>+</sup> accumulation on Ca, Mn, Fe and Cl<sup>-</sup> composition of *T. occidentalis*



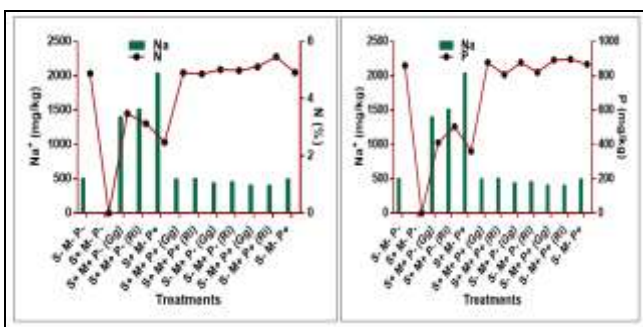
**Fig 5:** Comparative assessment of the influence of foliar Na<sup>+</sup> accumulation on K, Mg, Ca and Mn composition of *C. maxima*



**Fig 3:** Comparative assessment of the influence of foliar Na<sup>+</sup> accumulation on Zn composition and Na<sup>+</sup>/K<sup>+</sup> ratio of *T. occidentalis*



**Fig 6:** Comparative assessment of the influence of foliar Na<sup>+</sup> accumulation on Fe, Cl<sup>-</sup>, Zn composition and Na<sup>+</sup>/K<sup>+</sup> ratio of *C. maxima*



**Fig 4:** Comparative assessment of the influence of foliar Na<sup>+</sup> accumulation on N and P composition of *C. maxima*

In this study, it was observed that the K content of *C. maxima* and *T. occidentalis* under saline treatments was significantly ( $p=0.05$ ) reduced while there was a significant increase in Na<sup>+</sup> and Na<sup>+</sup>/K<sup>+</sup> ratio. Studies carried out by

Evelin *et al.* [24] revealed that NaCl treatment reduced the level of K<sup>+</sup>, an antagonist of Na<sup>+</sup>. Porcel *et al.* [32] reported increased accumulation of Na<sup>+</sup> in rice root than shoot tissues due to a decreased root-to-shoot distribution of Na<sup>+</sup> and suggested this to be one of the key strategies for alleviating salinity stress and maintaining plant growth under salt conditions. K plays a key role in plant metabolism. It is essential for activating a range of enzymatic reactions such as during the formation of pyruvate, stomatal activities, protein synthesis at the time of tRNA binding to the ribosomes and maintaining osmotic pressure of the vacuole and cell turgor [11]. These functions cannot be replaced by Na<sup>+</sup> ions [33]. Elevated Na<sup>+</sup> in the soil solution inhibits the uptake of other nutrients by interfering with various transporters in the root plasma membrane, such as K<sup>+</sup>-selective ion channels, and inhibition of root growth by the adverse effects of Na<sup>+</sup> on soil structure [3]. Salinity stress reduced the level of K<sup>+</sup>; an antagonist of Na<sup>+</sup>. Since Na<sup>+</sup> and K<sup>+</sup> have similar chemical properties, therefore cytoplasmic Na<sup>+</sup> competes for the similar binding sites and hence inhibits the metabolic process that depend on K<sup>+</sup>. A higher Na<sup>+</sup>/K<sup>+</sup> ratio caused by salinity is known to interrupt the cytoplasm ionic balance, and consequently inhibit various metabolic pathways [33, 34].

Root colonization of *C. maxima* and *T. occidentalis* by *R. irregularis* and *G. geosporum* significantly alleviated the K content of the study plants. However, Evelin *et al.* [24] observed that *G. intraradices* colonized plants had higher foliar and root K<sup>+</sup> concentrations compared to non-mycorrhizal plants under salinity stress showing the potential of mycorrhiza in preventing the disruption of K<sup>+</sup> homeostasis. The capacity of plants to maintain a high cytosolic K<sup>+</sup>/Na<sup>+</sup> ratio is one of the key determinants of plant salt tolerance [24]. The efficacy of *G. intraradices* in maintaining favorable K<sup>+</sup>/Na<sup>+</sup> ratio has also been reported by other authors [35, 36]. This may be explained by dilution effect resulting from plant growth enhancement by AMF colonization [34]. It is accomplished by regulating the expression and activity of K<sup>+</sup> and Na<sup>+</sup> transporters and of H<sup>+</sup> pumps that generate the driving force to transport ions [37]. The higher K<sup>+</sup>/Na<sup>+</sup> ratio helps to prevent the disruption of various K-mediated enzymatic processes and inhibition of protein synthesis. High K<sup>+</sup>/Na<sup>+</sup> ratios are also beneficial in influencing the ionic balance of the cytoplasm or Na<sup>+</sup> efflux from plants [24].

#### 4. Conclusion

Soil salinity is one of the most severe abiotic stresses affecting plant establishment, growth and production worldwide as observed in this study. Results of this study revealed that salt stress negatively affected physicochemical properties of the saline soil when compared to the garden soil, thus resulting in negative effects on minerals contents of *C. maxima* and *T. occidentalis*. The effects of mycorrhizal symbiotic association on *C. maxima* and *T. occidentalis* showed improvements on the mineral nutrition of the test plants. Using different mechanisms *C. maxima* and *T. occidentalis* by itself or in association with arbuscular mycorrhizal fungi and poultry manure (PM) can tolerate or survive soil salinity. However, in the presence of the fungi, plant ability to resist the stress increases as a result of physiological changes and improved vigour, adjusted rate of K<sup>+</sup>/Na<sup>+</sup>, extensive network of the mycorrhizal plant roots and enhanced nutrient uptake are all

among the processes that made the mycorrhizal inoculated plants to survive under salt stress.

#### References

1. Saxena B, Giri B, Shukla K. Arbuscular mycorrhizal fungi and tolerance of salt stress in plants. In: Q. S. Wu, (Editor), Arbuscular mycorrhizas and stress tolerance of plants. Springer Nature Singapore Pte Ltd, Singapore, 2017, 73p.
2. Kumar A, Sharma S, Mishra S. Influence of arbuscular mycorrhizal (AM) fungi and salinity on seedling growth, solute accumulation and mycorrhizal dependency of *Jatropha curcas* L. Journal of Plant Growth Regulator, 2010; 29:297-306.
3. Porcel R, Aroca R, Ruiz-Lozano JM. Salinity stress alleviation using arbuscular mycorrhizal fungi: a review. Agronomy for Sustainable Development, 2012; 32:181-200.
4. FAO (Food and Agricultural Organization). Integrated soil management for sustainable agriculture and food security in southern and east Africa. In: H. Nabhan, A. Mashali and A. R. Mermut (Edition) Proceedings of the expert consultation Harare, Zimbabwe, 1999, 12p.
5. Ruiz-Lozano JM, Porcel R, Azcón C, Aroca R. Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants: new challenges in physiological and molecular studies. Journal of Experimental Botany. 2012; 63(11):4033-4044.
6. Kumar A, Dames JF, Gupta A. Current developments in arbuscular mycorrhizal fungi research and its role in salinity stress alleviation: a biotechnological perspective. Critical Reviews in Biotechnology, 2014, 1-14.
7. Bothe H. Arbuscular mycorrhiza and salt tolerance of plants. Symbiosis. 2012; 58:7-16.
8. Maathuis FJM, Ahmad I, Patishtan J. Regulation of Na<sup>+</sup> fluxes in plants. Frontiers in Plant Science, 2014, 5:467.
9. Evelin H, Kapoor R, Giri B. Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Annuals of Botany, 2009; 104:1263-1280.
10. Smith SE, Read DJ. Mycorrhizal symbiosis. Academic Press, San Diego, California, 2008, 34p.
11. Evelin H, Giri B, Kapoor R. Contribution of *Glomus intraradices* inoculation to nutrient acquisition and mitigation of ionic imbalance in NaCl-stressed *Trigonella foenum-graecum*. Mycorrhiza, 2012; 22:203-217.
12. Evelin H, Giri B, Kapoor R. Ultrastructural evidence for AMF mediated salt stress mitigation in *Trigonella foenum-graecum*. Mycorrhiza, 2013; 23:71-86.
13. Asghari HR, Chittleborough DJ, Smith FA, Smith SE. Influence of arbuscular mycorrhizal (AM) symbiosis on phosphorus leaching through soil cores. Plant and Soil, 2005; 275:181-193.
14. Hajiboland R, Aliasgharzadeh N, Laiegh SF, Poschenreider C. Colonization with arbuscular mycorrhizal fungi improves salinity tolerance of tomato (*Solanum lycopersicum* L.) plants. Plant and Soil, 2010; 331:313-327.
15. AKSG. Geography and location about Akwa Ibom State. <http://www.aksg.online.com>. (Retrieved on 27th January 2017), 2008.

16. AOAC (Association of Official Analytical Chemists). Official Methods of Analysis. 10<sup>th</sup> and 17<sup>th</sup> Edition. Association of Official Analytical Chemists, Washington D. C, 2005, 98p.
17. AOAC (Association of Official Analytical Chemists). Official Methods of Analysis. 17<sup>th</sup> Edition. Association of Official Analytical Chemists, Arlington, Virginia, 2003, 105p.
18. Khan ZI, Hussain A, Ashraf M, Mc-Dowell LR. Mineral status of soil and forages in South Western Punjab, Pakistan. *Asian Journal of Animal Science*. 2006; 19:1139-1147.
19. Miller RW, Gardiner, DT. Soils in our environment. 9th Edition, Prentice Hall-Incorporated, Upper. Saddle River, New Jersey 07458, 2007, 452p.
20. Dedeké OA, Akomolafé GF. Influence of salinity on soil chemical properties and surrounding vegetation of an open-pit coal mining site, Nasarawa State, Nigeria. *African Journal of Environmental Science and Technology*. 2013; 7(12):1072-1075.
21. Garg N, Manchanda G. Effect of arbuscular mycorrhizal inoculation of salt-induced nodule senescence in *Cajanus cajan* (pigeon pea). *Journal of Plant Growth Regulation*, 2008; 27:115-124.
22. Robert MA, Heather DT, Arnold MS. Arbuscular mycorrhizal symbiosis and osmotic adjustment in response to NaCl stress: a meta-analysis. *Frontiers of Plant Science*, 2014; 5:3-4.
23. Ullah SM, Gerzabek MH, Soja G. Effect of seawater and soil salinity on onion uptake, yield and quality of tomato (fruit). *Die Bodenkultur*, 1994; 45:227-237.
24. Evelin H, Giri B, Kapoor R. Contribution of *Glomus intraradices* inoculation to nutrient acquisition and mitigation of ionic imbalance in NaCl-stressed *Trigonella foenum-graecum*. *Mycorrhiza*, 2011; 22:1-15.
25. Cantrell IC, Linderman RG. Preinoculation of lettuce and onion with VA mycorrhizal fungi reduces deleterious effects of soil salinity. *Plant and Soil*, 2001; 233:269-281.
26. Silveira JAG, Melo ARB, Viegas RA, Oliveira JTA. Salinity induced effects on nitrogen assimilation related to growth in cowpea plants. *Environmental and Experimental Botany*, 2001; 46:171-179.
27. Colla G, Roupael Y, Cardarelli M, Tullio M, Rivera C, Rea E. Alleviation of salt stress by arbuscular mycorrhizal in zucchini plants grown at low and high phosphorus concentration. *Biology and Fertility of Soils*. 2008; 44:501-509.
28. Miransari M. Arbuscular mycorrhizal fungi and heavy metal tolerance in plants. In: Q. S. Wu (Editor.), *Arbuscular mycorrhizas and stress tolerance of plants*. Springer Nature Singapore Pte Ltd, Singapore, 2017, 150p.
29. Giri B, Kapoor R, Mukerji KG. Influence of arbuscular mycorrhizal fungi and salinity on growth, biomass and mineral nutrition of *Acacia auriculiformis*. *Biology and Fertility of Soils*. 2003; 38:170-175.
30. Sheng M, Tang M, Chan H, Yang B, Zhang F, Huang Y, *et al.* Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. *Mycorrhiza*, 2008; 18:287-296.
31. Abdel LAA, Chaoxing H. Effect of arbuscular mycorrhizal fungi on growth, mineral nutrition, antioxidant enzymes activity and fruit yield of tomato grown under salinity stress. *Science Horticulture*, 2011; 127:228-233.
32. Porcel R, Aroca R, Azcon R. Regulation of cation transporter genes by the arbuscular mycorrhizal symbiosis in rice plants subjected to salinity suggests improved salt tolerance due to reduced Na<sup>+</sup> root-to-shoot distribution. *Mycorrhiza*, 2016; 26:673.
33. Giri B, Kapoor R, Mukerji KG. Improved tolerance of *Acacia nilotica* to salt stress by arbuscular mycorrhiza, *Glomus fasciculatum*, maybe partly related to elevated K<sup>+</sup>/Na<sup>+</sup> ratio in root and shoot tissues. *Microbial Ecology*, 2007; 54:753-760.
34. Hajiboland R. Role of arbuscular mycorrhiza in amelioration of salinity. In: P. Ahmad (Editor). *Salt stress in plants: signaling, omics and adaptations*. Springer, New York, 2013, 354p.
35. Porras-Soriano A, Soriano-Martin ML, Porras-Piedra A, Azcon R. Arbuscular mycorrhizal fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions. *Journal of Plant Physiology*. 2009; 166:1350-1359.
36. Shokri S, Maadi B. Effects of arbuscular mycorrhizal fungus on the mineral nutrition and yield of *Trifolium alexandrinum* plants under salinity stress. *Journal of Agronomy*, 2009; 8:79-83.
37. Parida SK, Das AB. Salt tolerance and salinity effects on plants. *Ecotoxicology and Environment Safety*, 2005; 60:324-349.