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Growth and Some Physiological Attribute Responses of *Cucurbita maxima* Duch. to Sea Salt Stress and Mycorrhizal Fungi Mitigation

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Abstract
The current research was conducted to examine the role of arbuscular mycorrhizal fungi (*Rhizophagus irregularis*) in alleviating adverse effects of sea salt water and saline soil stress in *Cucurbita maxima.* Physicochemical properties of the experimental soils (saline and garden soils) and irrigation water (sea and fresh water) indicated significant (p=0.05) differences between the two soil types in; pH, total nitrogen, available phosphorus, Ex. Na and in irrigation water; EC as well as salinity, Cl^-, Na^+ and EC. Saline soil/water treatment reduced photosynthetic pigments (chlorophyll a, b and carotenoids), minerals, relative water content, biomass yield as well as percentage mycorrhizal root colonization (45.45 to 20.34%) and mycorrhizal dependency (100.00% to 13.87%). The symbiotic association between *R. irregularis* with roots of *C. maxima* showed improvements on the biomass and physiological attributes of *C. maxima* through morphological and physiological vicissitudes and improved vigour to survive under severe salt stress conditions.

Keywords: *Cucurbitaceae*, *Glomeraceae*, Mycorrhiza, *Rhizophagus irregularis*, Salinity, Sea salt

1. Introduction
The constant build-up of salts in the soil is one of the major coercions facing crop production worldwide [1, 2]. According to estimates; about 7% of the earth's agriculturally useful land is exposed to high soil salinity levels [2]. Salinity may be regarded as the content of water-soluble salts, predominantly sodium, potassium, magnesium, calcium and chloride, in the soil. A soil that possesses an electrical conductivity of a saturated paste extract (ECe) values of about 4 dS/m or more, which is comparable to 40 mM of NaCl, are distinctive of saline soils [3]. Na^+, Ca^{2+}, Mg^{2+}, and K^+ are amongst the major cations found in saline soils; whereas, the major anions include Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-} and NO_3^-. These constituents are usually reported in units of mg/L (ppm), mmol/L or mmol charge/L (meq/L) in solution extracted from a soil saturated with water [4]. The negative impact of salinity not only declines the agricultural production of most crops, but also, but also has a serious effect on soil physicochemical properties, which adversely affects the associated ecological balance of the area in question. Salinity affects crop production in various ways including: low agricultural production, low economic returns due to high cost of cultivation, reclamation management, soil erosion due to high dispersibility of soil, ecological imbalance due to halophytes and marine life forms from fresh water to brackish water, poor human health due to toxic effects of elements such as B, F, and Se [5].

The model plant in this experiment is *Cucurbita maxima* Duchesne which belongs to the family *Cucurbitaceae* and is a coarse, prostrate or climbing, annual, herbaceous vine, reaching a length of 4 meters or more. Leaves are hispid, rounded, 15 - 30 centimeters in diameter, heart-shaped at the base, shallowly 5-lobed, with finely toothed margins, and often mottled on the upper surface. Flowers are bell-shaped, erect, yellow and about 12 centimeters long. Fruit is large, variable in shape, fleshy, with a yellow pulp. Seeds are ovoid or oblong, compressed, and about 1.3 centimeters long [6]. *C. maxima* varieties are used for preparation of many dishes. In Cameroon, Nigeria and other Western African countries, seeds of *C. maxima* are widely used as a vegetable, roasted and salted, or ground into a thick paste that is mixed with vegetables in cooking [7]. The medicinal uses of *C. maxima* include the dried pulp, in the form of confection, used as remedy for hemoptysis and hemorrhages from the pulmonary tract [6].

Beneficial microorganisms such as arbuscular mycorrhizal fungi (AMF) are able to colonize plants in their natural environment. AM fungi have been shown to promote plant growth and salinity tolerance by many researchers. Some microorganisms, particularly beneficial bacteria and fungi can result in improved plant performance under severe or combinational stress environments and consequently enhance yield [8]. Over 80 % terrestrial plant species is said to form symbiotic associations with AM fungi [9]. *Rhizophagus irregularis* belongs to the family *Glomeraceae*. Spores colour of *R. irregularis* is white, cream, and yellow-brown. Shape is elliptical with irregularities. Size is generally between 40 - 140 µm, Hyphae shape is Cylindrical or slightly flared [10]. Size - Width: 11 - 18 µm [10]. In numerous scientific studies *R. irregularis* has been shown to increase phosphorus uptake in multiple plants as well as improve soil aggregation due to hyphae; because of these qualities, *R. irregularis* is commonly found in mycorrhizal based fertilizers. *R. irregularis* colonization peaks earlier than many of the other fungi in the *Glomus* genus [10]. There tends to be extensive hyphal networking and intense intraradical spores associated

with older roots of host plants. At times the spores are densely clustered or patchily distributed, depending on the host species. When the spores are heavily clustered, mycorrhizologists and others will tend to mistake *Rhizophagus irregularis* for *Glomus fasciculatum* [10].

2. Materials and Methods

Sample Collection Site

Saline soil and salt water were collected from the saline ecosystem of Iwuochang community, 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 [11]. 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 *Rhizophagus irregularis* inoculum (60 – 65 spores per 5 g) was purchased from International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

Table 1: Experimental design

Treatments	Meaning
S- M-	- Salinity, - Mycorrhiza
S+ M-	+ Salinity, - Mycorrhiza
S+ M+	+ Salinity, + Mycorrhiza (*Rhizophagus irregularis*)
S- M+	- Salinity, + Mycorrhiza

Physico-chemical Properties of Experimental Soils

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 [12] procedure for wet acid digestions.

Analysis of Water Samples

Water pH, EC and TDS was measured using portable pH / EC / TDS / Temperature combined HANNA, HI 991301 model instrument. Other parameters were determined according to the standard procedures outlined by the Association of Official Analytical Chemist [12].

Estimation of Photosynthetic Pigments

The photosynthetic pigments (chlorophyll a [Chl a], chlorophyll b [Chl b], and carotenoids,) were extracted from leaves of *C. maxima* plants in dimethyl sulfoxide (DMSO) as described by Hiscox and Israelstam [13]. Absorbance was determined spectrophotometrically at 480, 510, 645, and 663 nm (T80 UV/VIS Spectrometer, PG Instruments Ltd, USA). DMSO was used as blank.

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

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), 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 [14].

Determination of Shoot Length
Measurement of shoot length was taken at nine (9) weeks after planting following seedling emergence using a measuring tape.

Determination of Leaf Area
Measurements were obtained using graph paper (grid method). The area (A) of the leaf was determined by tracing the outlines of the leaves on a standard graph paper. The area covered by the outline was then calculated (one small square on the graph represents 1 cm^2). The correlation factor (K) was determined by dividing the area (A) by product of length x breadth of the leaf. Thereafter, the leaf area for each plant was determined using the formula:

$$A = L \times B \times K$$

Where: A = the traced area, L = Leaf length, B = Leaf width, K = Correction factor

Determination of Leaf Dry Weight
Leaf dry weight was determined after oven drying the leaves to constant weight at 70°C

Leaf Relative Water Content
Two (2) young fully expanded leaves were collected from each of two plants per replicate. Individual leaves were first detached from the stem and then weighed to determine the fresh weight (FW). To determine the turgid weight (TW), leaves were floated in distilled water inside a closed petri dish. Leaf samples were weighed periodically after gently wiping the water from the leaf surface with tissue paper until a steady state was achieved. At the end of the imbibition period, leaf samples were placed in a preheated oven at 80°C for 48 hours to determine the dry weight (DW). Values of FW, TW, and DW were used to calculate LRWC using the formula [15]:

$$LRWC\,(\%) = \frac{(FW - DW)}{TW - DW} \times 100$$

Quantification of Arbuscular Mycorrhizal Colonization in Plant Roots
Feeder roots of about 2 – 4 cm of *T. occidentalis* were separately collected, fixed in 50% ethanol and stored for colonization assessment. The fixed roots were rinsed in tap water before clearing in 10% KOH w/v and autoclaved for about 15 minutes at 121 ^{0}C. Cleared roots were collected on a fine sieve and rinsed with water several times before being transferred into the staining solution. Staining of the plants roots was carried out using 5% ink diluted in vinegar (5% acetic acid). The roots segments were soaked in the ink and left in staining solution at room temperature for one day. Stained roots were later destained in 50% glycerol for 1 hour [16].
Stained roots were randomly dispersed in a 9 cm diameter Petri plate with grid lines. Vertical and horizontal gridlines were scanned at x40 magnification with a dissecting microscope. The proportion of root length that is mycorrhizal and total root length can then be calculated from a conversion factor derived from the total length of grid lines and the area of the dish. A minimum of 100 intersections was used to assess the stained root samples; the samples were re-randomized and counted several times. Mycorrhizal root colonization was thus determined by estimation of percentage of root segments containing hyphae, arbuscules and vesicles [17].

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

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

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

Statistical Analysis
The study was conducted using complete randomized design with three (3) replicates for each plant. 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 (± 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 Discussion

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). 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 [18] who reported an increase in pH and EC in saline soils in New Jersey due to salt stress. Deleke and Akomolafe [19] 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. Soil organic carbon content is influenced by two opposing factors: reduced plant inputs and reduced rates of decomposition [20].

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

Irrigation Water (Saline and Freshwater) Analysis

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, sulphate, nitrate, Cl^-, Ca^{2+}, Mg^{2+}, Na^+, K^+ and salinity. There was no significant (p=0.05) difference in acidity, DO, BOD and phosphate (Table 3). Freshwater can easily be absorbed by plants without any difficulty; sea salt water absorption tends to pose serious difficulty to plants depending on the salinity tolerance level of such plant. When plants are exposed to high saline water, they expend too much energy trying to absorb water against concentration gradient, thus limiting the moisture content of the plant and subsequently reduction in growth and productivity,

Table 3: Water analysis of the experimental irrigation water

S.No.	Parameters	Saline Water	Freshwater	t-values
1.	pH	7.70	6.70	3.273
2.	EC (µS/cm)	3080.00	27.70	13.063*
3.	TDS	1021.00	11.00	7.063*
4.	Acidity (mg/l as $CaCo_3$)	80.00	95.40	-1.130
5.	Alkalinity (mg/l as $CaCo_3$)	138.00	53.20	76.468*
6.	DO (mg/L)	6.40	7.60	-3.082
7.	BOD (mg/L)	3.20	2.80	1.852
7.	Sulphate (mg/L)	102.31	1.91	22.776*
9.	Phosphate (mg/L)	0.09	0.04	2.774
10.	Nitrate (mg/L)	0.06	2.82	-9.816*
11.	Cl^- (mg/L)	2560.13	55.23	30.446*
12.	Ca^{2+} (mg/L)	55.71	106.20	-8.870*
13.	Mg^{2+} (mg/L)	120.20	232.81	13.544*
14.	Na^+ (mg/L)	1027.00	0.11	16.945*
15.	K^+ (mg/L)	6.42	8.40	11.609*
16.	Salinity (ppt)	33.21	0.32	20.839*

* Significant at t = 0.05, EC – Electrical conductivity, DO – Dissolved oxygen, BOD – Biological oxygen demand, TDS – Total dissolved solids

Soil salinity, sea saline water irrigation and AMF inoculation on shoot length and leaf area of Cucurbita maxima

Growth parameters of *C. maxima* and *T. occidentalis* such as leaf area and shoot length were all significantly (p=0.05) reduced with sea salt water treatments when compared to the control at 3 weeks (Figure 2 and 3). Inoculation with AMF however increased the growth parameters of *C. maxima* in spite of sea salt water and soil treatments (Figure 2 and 3). However, *C. maxima* plants died after the sixth (6) weeks of sea salt water saline soil treatments. Similar findings have been reported with *Vigna aconitifolia* L. [21], *Raphanus sativus* L. [22], *Vigna unguiculata* L. [23] and *Vigna mungo* L. [24]. They reported that increased salinity results in a decline in the shoot lengths of the plants. Salt stress has also been reported to cause significant reduction in leaf area in *Vicia faba* [25], *V. aconitifolia* L. [26], *Avena sativa* L. [27] and *Fragaria xananssa* L. [28]. This notable decrease in leaf area in saline treatments in this study is as a result of the increased concentrations of Na^+ and Cl^-. This could be explained by the negative effect of salt on photosynthesis and subsequently reduction in plant growth, leaf growth to avoid escape of water via transpiration and chlorophyll content [29].

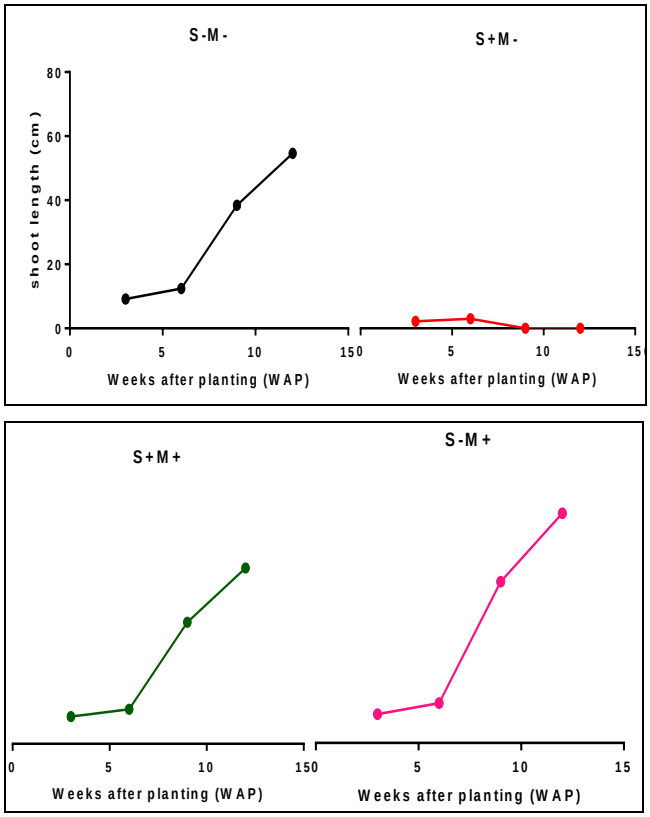

Fig 2: The effect of soil salinity, sea saline water irrigation and AMF inoculation on shoot length of *Cucurbita maxima*

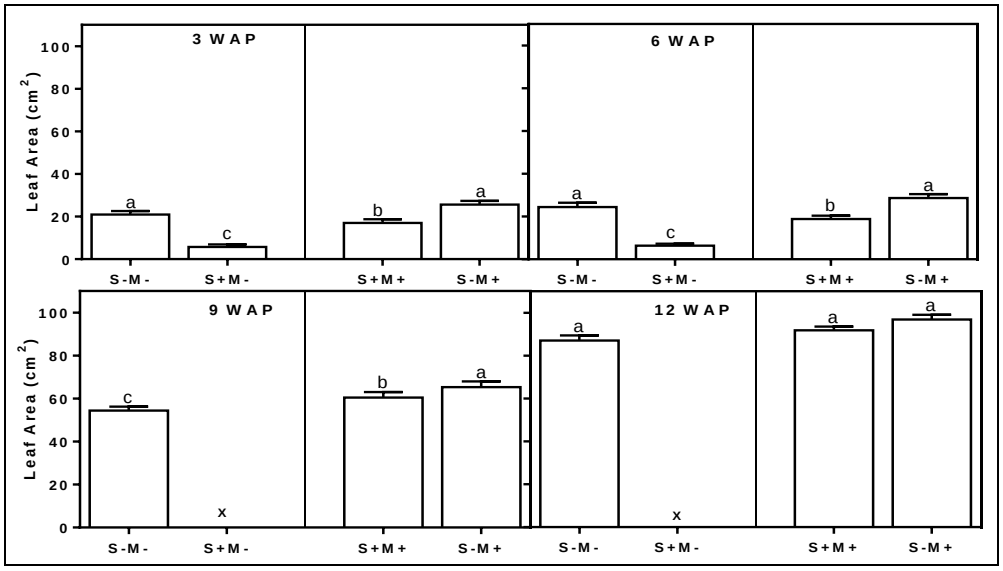

Fig 3: The effect of soil salinity, sea saline water irrigation and AMF inoculation on leaf area of *Cucurbita maxima*

Soil salinity, sea saline water irrigation and AMF inoculation on photosynthetic pigments of Cucurbita maxima

The content of photosynthetic pigments of *C. maxima* (such as chlorophyll a, chlorophyll b and carotenoids) grown in saline soils and irrigated with sea saline water taken at 9 WAP were significantly (p=0.05) reduced when compared to the control while inoculation with AMF (*R. irregularis*) significantly (p=0.05) increased these pigments in the test plant (Figure 4). Amira and Abdul [25] recorded decrease in chlorophyll 'a', 'b', and total chlorophyll in *V. faba* exposed to saline stress. Also, Tort and Turkyilmaz [30] reported that the exposure of barley (*Hordeum vulgare* L.) to several levels of salinity led to decrease in chlorophyll 'a', chlorophyll 'b' and total chlorophyll content. Under stress conditions, the photosynthetic activity of the plant is often affected due to the alteration in the level and activity of various photosynthetic enzymes such as rubisco [31], sucrose phosphate synthase and fructose-1,6-bisphosphatase [32], and PEP carboxylase [33]. All these could have led to reduction in photosynthesis and subsequent lower biomass accumulation resulting in growth reduction in saline soil grown plants. The influence of AMF on photosynthesis has been reported in many mycorrhizal plants growing under salinity stress [34, 35]. The increased rate of photosynthesis in AMF-colonized plants under salinity stress has been correlated with lower intercellular CO_2 concentration in mycorrhizal plants, since the higher photosynthetic capacity increases water use efficiency for the assimilation of more carbon per unit water transpiration [36].

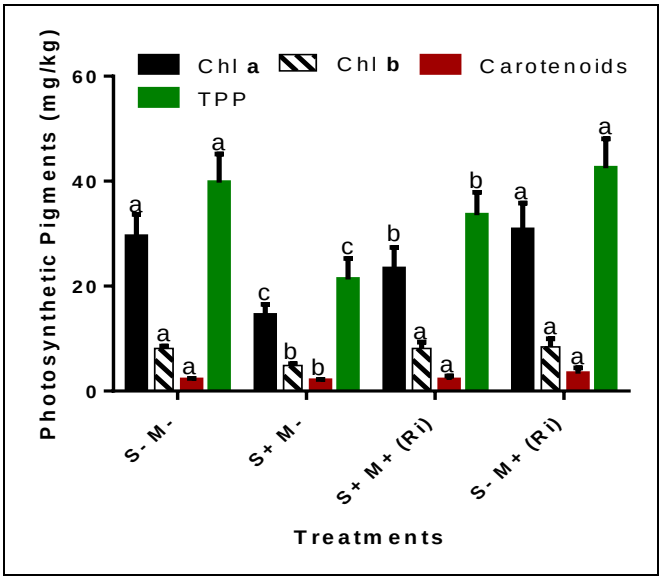

Fig 4: Effects of AMF inoculation on the photosynthetic pigments content of *C. maxima* grown in saline soil and irrigated with sea saline water**.**
[a]Means within of each coloured bar followed by different letters are significantly different at p=0.05 according to Duncan's Multiple Range Test. S- (No salinity), M- (No mycorrhiza), S+ (Plus salinity), M+ (Plus mycorrhiza), (*Ri*) – *Rhizophagus irregularis.*

Impact of Arbuscular Mycorrhizal Fungi (AMF) Inoculation on the Mineral Nutrient Contents and Biomass Yield of C. maxima Grown in Saline Soil and irrigated with sea saline water

Mineral nutrient contents of *C. maxima* P, K, Mg and Ca were significantly (p=0.05) reduced in saline water/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 with AMF (*R. irregularis*) significantly (p=0.05) increased the mineral nutrient contents, leaf relative water content and biomass yield in the test plant in saline and non-saline soil/water treatments (Table 4). The mineral (N, P, K, Mg and Ca), leaf relative water content and biomass yield of *C. maxima* were significantly (p=0.05) reduced in saline soil treatments in 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 minerals, leaf relative water content and biomass yield, it was observed that Na^+ accumulation had negative effects on the minerals, leaf relative water content and biomass yield of *C. maxima* (Figure 5 and 6). This observation agrees with the work of Robert *et al*. [37] 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^+ ions. Ullah *et al.* [38] reported that irrigation of tomato plants with seawater increased the uptake of sodium chloride and decreased the uptake of P. Increased concentration of Na^+ and Cl^- in soil solution competes with the uptake of vital ions such as Ca^{+2}, P, K^+, Mg^{+2}, and N and alters the ideal salt ratios in the soil solution, therefore affecting plant nutrient acquisition and restricting plant growth and biomass. In this study, it was also observed that the minerals composition of *C. maxima* under saline and non-saline treatments was significantly increased with AMF *R. irregularis* inoculation. A higher N, P, K, Mg and Ca concentration in mycorrhizal than non-mycorrhizal plants can favourably alleviate the toxic effects of NaCl by inducing a higher K^+/Na^+ rate leading to salt adaptation [39]. Cantrell and Linderman [40] reported increased N, P, K, Mg and Ca uptake in mycorrhizal lettuce.

Table 4: Impact of arbuscular mycorrhizal fungi (AMF) inoculation on the mineral nutrient contents of *C. maxima* grown in saline soil and irrigated with sea saline water

Parameters	S- M-	S+ M-	S+ M+ (*Ri*)	S- M+ (*Ri*)
N (%)	*5.03^a	0.00	3.44^b	5.28^a
P (mg/kg)	754.10^b	0.00	510.10^c	894.40^a
K (mg/kg)	4610.10^b	0.00	2215.10^c	4811.10^a
Mg (mg/kg)	560.14^b	0.00	418.00^c	601.10^a
Ca (mg/kg)	3008.10^b	0.00	1620.20^c	3240.00^a
Na (mg/kg)	387.70^b	0.00	1320.20^a	309.10^c
Na^+/K^+ ratio	0.08^b	0.00	0.59^a	0.06^b
Leaf Relative Water Content (LRWC) (%)	*38.00 ± 2.51^a	0.00	36.36 ± 1.66^b	42.65 ± 1.57^a
Total Dry Weight (gplant^{-1})	*5.06^a	0.00	3.18^b	6.43^a

*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), (*Ri*) – *Rhizophagus irregularis*, 0.00 (Plants died off)

Percentage AMF root colonization of *C. maxima* was significantly (p=0.05) reduced in saline soil treatments of the test plants when compared to the non-saline inoculated treatments (Table 5). However, inoculated saline soil treatment showed significant (p=0.05) increased in mycorrhizal dependency compared to the non-stressed inoculated treatment. This observation is in agreement with earlier studies reporting that addition of salt to soil inhibits hyphal growth, which subsequently reduces the spread of mycorrhizal colonization [41, 42]. It was also observed in this study that mycorrhizal dependency (MD) varied with saline and non-saline treatments. Root colonization of *C. maxima* by *R. irregularis* showed great dependency of these plants on the AMF when compared to the purely non-saline mycorrhizal treatment. This corroborates the work of Beltrano *et al.* [43] who demonstrated the favourable relationship between pepper and *G. intaradices* (*R. irregularis*), and showed that when roots were associated with AM fungi, the detrimental effect of the salinity stress decreased significantly. Taken together, although salinity reduced mycorrhizal colonization, the dependency of pepper plants on mycorrhizal fungi was high [43].

Table 5: Arbuscular mycorrhizal fungi (AMF) root colonization of *C. maxima* grown in saline soil and irrigated with sea saline water

Non-inoculated treatment	Root colonization (%)	Mycorrhizal Dependency (%)	Inoculated treatments	Root colonization (%)	Mycorrhizal Dependency (%)
S-M-	0.00	0.00	S+M+ (*Ri*)	*20.34^b	100.00^a
S+M-	0.00	0.00	S-M+ (*Ri*)	45.45^a	13.87^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), (*Ri*) – *Rhizophagus irregularis*.

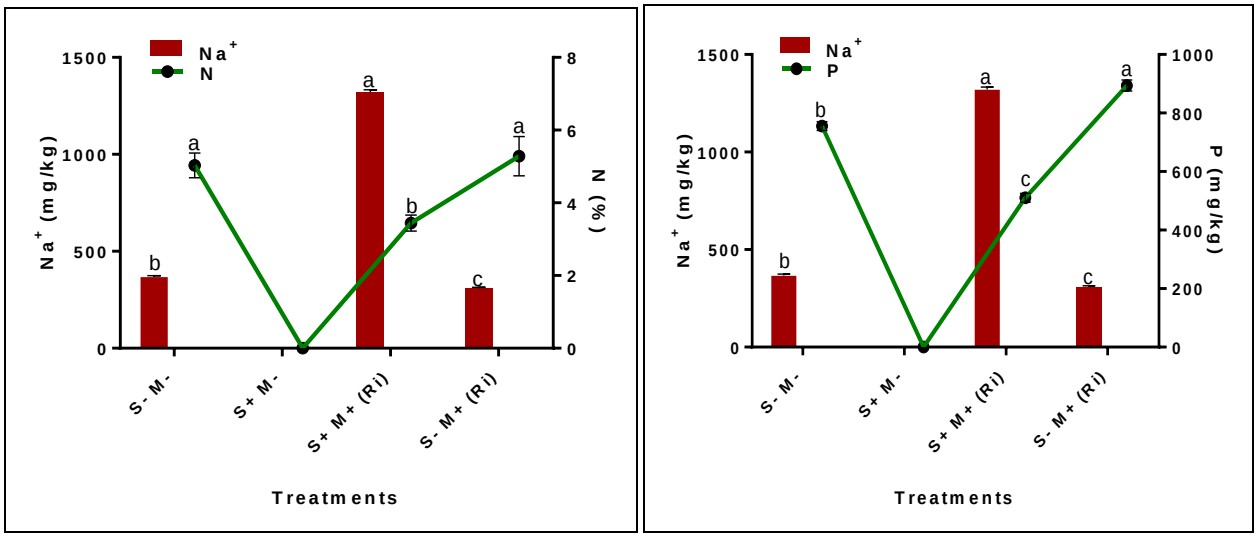

Fig 5: Influence of AMF inoculation on foliar Na^+ accumulation in *C. maxima* as it affects its N and P uptake

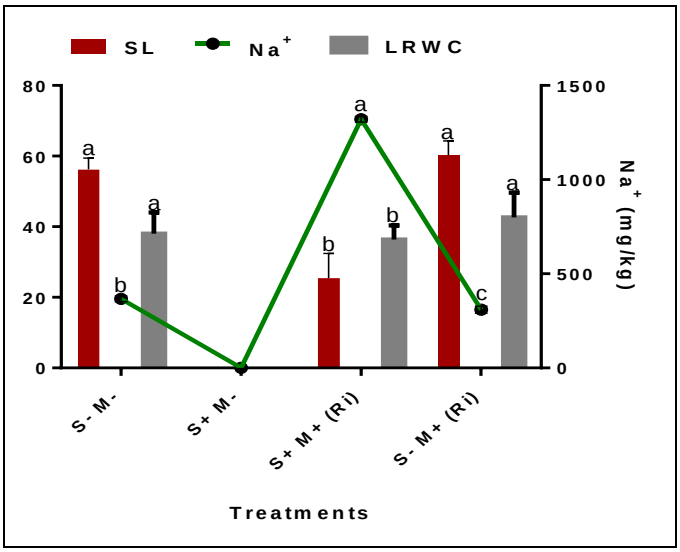

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Fig 6: Influence of AMF inoculation on foliar Na^+ accumulation in *C. maxima* as it affects its Shoot length (SL) and Leaf relative water content (LRWC) content

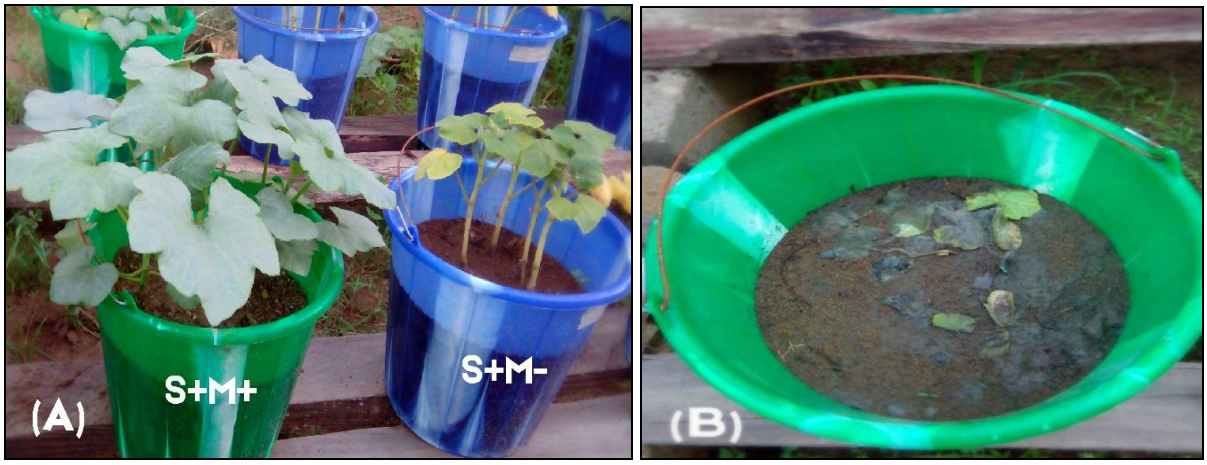

Fig 7(A): Shows growth response of *C. maxima* to *Rhizophagus irregularis* inoculation in saline treatment (S+M+) and uninoculated saline treated plants (S+M-) six (6) weeks after planting. **(B):** Uninoculated saline treated (S+M-) *C. maxima* dies off twelve (9) weeks after planting; soil shows hard surface crusting which results in poor soil aeration, porosity and low microbial activities.

4. Conclusion

Sea water intrusion into farmlands depositing soluble salts and the accumulation of such salts in the soil is one of the major threats facing crop production and yield globally. Documented estimates have it that about 7% of the earth's land is exposed to high soil salinity levels. Results of the comparative physicochemical properties of the experimental soils and water used for this study revealed that the saline water and soil possessed certain characteristics (such as high pH, EC, salinity, Ex.Na etc) which does not support proper

crop growth and survival when compared to the garden soil, thus resulting in negative effects on photosynthetic pigments, minerals contents, relative leaf water content (RLWC), shoot length, dry weight, mycorrhizal colonization and dependency of *C. maxima*. The effects of mycorrhizal symbiotic association on *C. maxima* showed improvements on the growth and physiology of *C. maxima*. Using different mechanisms *C. maxima* by itself or in association with arbuscular mycorrhizal fungi (*R. irregularis*) can tolerate or survive soil salinity. However, symbiotic association of the fungus *R. irregularis* with roots of *C. maxima* gives the plant the ability to resist the stress as a result of morphological and physiological vicissitudes and improved vigour/water content, adjusted rate of K^+/Na^+, extensive network of the mycorrhizal plant roots and enhanced nutrient uptake are all among the processes that made the mycorrhizal inoculated plant to survive under severe salt stress conditions.

5. References

1. Nasim G. The role of arbuscular mycorrhizae in inducing resistance in drought and salinity stress in crops. Plant adaptation and phytoremediation, 2010; 1:119-141.
2. Ebrahim KE. *Role of arbuscular mycorrhizal fungi in fighting soil salinity*. PhD Thesis. Royal Holloway-University of London, London, England. 2014; 45p.
3. US Department of Agriculture. *Research database. Bibliography on salt tolerance. In*: George E. Brown, Fr. Salinity Lab. US department of agriculture. Serv. Riverside, CA. 2008; 24p. http://www.Ars.usda.gov/Services/docs.htm?docid=8908. (Retrieved on 26th August 2008).
4. Tanji KK. Salinity in the soil environment. *In*: Läuchli, A, Lüttge, U, editors. *Salinity: environment-plants-molecules*. Netherlands: Kluwer Academic Publishers. 2002; 21-51.
5. Ashok A, Nisha K, Karishma N, Anju T, Gupta KK. Arbuscular mycorrhizal symbiosis and alleviation of salinity stress. Journal of Applied and Natural Science. 2012; 4(1):144-155.
6. Villasenor IM, Bartolome ALO. Microbiological and pharamcological studies on extracts of Cucurbita maxima. Phytotherapy Research. 1995; 9(5):376-378.
7. Ngwerume FC, Grubben GJH. *Cucurbita maxima* Duchesne*:* Record from PROTA. G. J. H. Grubben and O. A. Denton (Editors) *Plant resources of tropical Africa (PROTA)*, Wageningen, Netherlands, 2004; p124.
8. Evelin H, Kapoor R, Giri B. Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Annuals of Botany, 2009; 104:1263-1280.
9. Smith SE, Read DJ. *Mycorrhizal symbiosis*. Academic Press, San Diego, Califonia, 2008; 34p.
10. Krüger M, Claudia K, Christopher W, Herbert S. and Arthur S. Phylogenetic Reference Data for Systematics and Phylotaxonomy of Arbuscular Mycorrhizal Fungi from Phylum to Species Level. New Phytologist, 2012; 193(193):970-984.
11. AKSG. Geography and location about Akwa Ibom State. http://www.aksg.online.com. (Retrieved on 27th January 2017) 2008.
12. AOAC (Association of Official Analytical Chemists). *Official Methods of Analysis*. 10th and 17th Edition. Association of Official Analytical Chemists, Washington D. C. 2005; 98p.
13. Hiscox JD, Israelstam GF. A method for the extraction of chlorophyll from leaf tissue without maceration. Canadian Journal of Botany. 1979; 57:1332-1334.
14. AOAC (Association of Official Analytical Chemists). *Official Methods of Analysis*. 17th Edition. Association of Official Analytical Chemists, Arlington, Virginia. 2003; 105p.
15. Kaya C, Kirnak H, Higgs D, Saltati K. Supplementary calcium enhances plant growth and fruit yield in strawberry cultivars grown at high (NaCl) salinity. *Science Horticulture*, 2003; 26:807-820.
16. Walker C. A simple blue staining technique for arbuscular mycorrhizal and other root-inhibiting fungi. Inoculum, 2005; 56(4):68-69.
17. Giovannetti M, Mosse B. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytologist. 1980; 84(3):489-500.
18. Miller RW, Gardiner DT. *Soils in our environment*. 9th Edition, Prentice Hall-Incorporated, Upper. Saddle River, New Jersey 07458, 2007; 452p.
19. Dedeke OA, Akomolafe GF. Influence of salinity on soil chemical properties and surrounding vegetation of awe salt mining site, Nasarawa State, Nigeria. African Journal of Environmental Science and Technology. 2013; 7(12):1072-1075.
20. 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.
21. Mathur N, Singh J, Bohra S, Bohra A, Vyas A. Biomass production, productivity and physiological changes in moth bean genotypes at different salinity levels. American Journal of Plant Physiology. 2006; 1(2):210-213.
22. Jamil M, Rehman SU, Lee KJ, Kim JM, Rha HK. Salinity reduced growth ps2 photochemistry and chlorophyll content in radish. *Science Agriculture* (Piracicaba, Braz.). 2007; 64(2):111-118.
23. Taffouo VD, Kouamou JK, Ngalangue LMT, Ndjeudji, BAN, Akoa A. Effects of salinity stress on growth, ions partitioning and yield of some cowpea (*Vigna ungiuculata* L. Walp) cultivars. International Journal of Botany. 2009; 5(2):135-143.
24. Kapoor K, Srivastava A. Assessment of salinity tolerance of *Vinga Mungo* var. pu-19 using ex vitro and in vitro methods. Asian Journal of Biotechnology. 2010; 2(2):73-85.
25. Amira MS, Abdul Q. Effect of salt stress on plant growth and metabolism of bean plant *Vicia faba* (L.). Journal of the Saudi Society of Agricultural Sciences. 2010; 10:7-15.
26. Mathur N, Singh J, Bohra S, Bohra A, Vyas A. Biomass production, productivity and physiological changes in moth bean genotypes at different salinity levels. American Journal of Plant Physiology. 2006; 1(2):210-213.
27. Zhao GQ, Ma BL, Ren CZ. Growth, gas exchange, chlorophyll fluorescence and ion content of naked oat in response to salinity. Crop Science, 2007; 47(1):123-131.
28. Yilmaz H, Kina A. The influence of nacl salinity on

some vegetative and chemical changes of strawberries (*Fragaria xananassa* L.). African Journal of Biotechnology. 2008; 7(18):3299-3305.
29. Netondo GW, Onyango JC, Beck E. Crop physiology and metabolism sorghum and salinity ii – gas exchange and chlorophyll fluorescence of sorghum under salt stress. *Crop Science*, 2004; 44(3):806-811.
30. Tort N, Turkyilmaz B. A physiological investigation on the mechanisms of salinity tolerance in some barley culture forms. Journal of Food Security. 2004; 27:1-16.
31. Valentine AJ, Mortimer PE, Lintnaar M, Borgo R. Drought responses of arbuscular mycorrhizal grapevines. Symbiosis, 2006; 41(3):127-133.
32. Ghosh S, Bagchi S, Majumder AL. Chloroplast fructose-1, 6-bisphosphatase from *Oryza* differs in salt tolerance property from the *porteresia* enzyme and is protected by osmolytes. Plant Science, 2001; 160:1171-1181.
33. Abdel LAA. Phosphoenol pyruvate carboxylase activity of wheat and maize seedlings subjected to salt stress. Australian Journal of Basic and Applied Science. 2008; 2:37-41.
34. 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.
35. Wu QS, Zon YN, Liu W. Alleviation of salt stress in citrus seedlings inoculated with mycorrhiza: changes in leaf antioxidant defense systems. Plant Soil Environment, 2010; 56:470-475.
36. Sheng M, Tang M, Chan H, Yang B, Zhang F, Huang Y. Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. Mycorrhiza, 2008; 18:287-296.
37. 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.
38. Ullah SM, Gerzabek MH, Soja G. Effect of seawater and soil salinity on ion uptake, yield and quality of tomato (fruit). Die Bodenkultur, 1994; 45:227-237.
39. 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.
40. 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.
41. Jahromi F, Aroca R, Porcel R, Ruiz-Lozano JM. Influence of salinity on the in vitro development of *Glomus intraradices* and on the in vivo physiological and molecular responses of mycorrhizal lettuce plants. Microbial Ecology, 2008; 55:45-53.
42. 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.
43. Beltrano J, Ruscitti M, Arango MC, Ronco M. Effects of arbuscular mycorrhiza inoculation on plant growth, biological and physiological parameters and mineral nutrition in pepper grown under different salinity and p levels. Journal of Soil Science and Plant Nutrition. 2013; 13(1):123-141.