

Poultry Manure and Arbuscular Mycorrhizal Fungi Synergy Improves Saline Soil Properties, Growth and Physiology in *Cucurbita maxima* Duch.

ABSTRACT

Aims: To assess the potential impacts of poultry manure (PM) amendment on saline soil properties and its synergy with arbuscular mycorrhizal fungi (AMF) on biomass yield and survival of *C. maxima*

Study design: The experiment was set up in complete block design of four (4) treatments with three (3) replicates

Place and Duration of Study: Soil samples were obtained from the saline ecosystem of Iwuochang (Latitude 4.56°N and Longitude 7.57°E), Akwa Ibom State, Nigeria. All analysis was carried out in Soil Science Laboratory and Botany Laboratory, Akwa Ibom State University, between January and march 2019.

Methodology: Soil samples were analyzed following the standard procedures outlined for wet acid digestions. Growth parameters were determined using standard methods. Leaf chlorophyll meter was employed in the assessment of the photosynthetic pigments of the experimental plant. Electrolyte leakage was assessed using the HANNA instrument conductivity meter. Leaf relative water content (LRWC), vigour index (VI) and plant salt tolerant index (PSTI) was calculated using standard formulas.

Results: Physicochemical analysis of the saline and poultry manure augmented soils indicated significant ($P = .05$) difference between the two soil types in pH, available phosphorus, total nitrogen, clay, sand, Ex. Ca, Mg, K, Na, OC and EC. Reduction in shoot length, number of leaves and leaf area as well as Chl a, b, carotenoids and total photosynthetic pigments (from 39.7 to 21.30mg/kg⁻¹) of *C. maxima* were all significantly ($P = .05$) affected by soil salinity stress. PSTI was significantly reduced while EL increased in saline soil treatments. Inoculation with AMF alone or together with PM significantly ($P = .05$) increased the growth parameters, photosynthetic pigments and physiological parameters in *C. maxima* both in saline and non-saline soil treatments.

Conclusion: The results of this work have shown that AMF and PM synergy can enhance the ability of *C. maxima* to resist salt stress possibly through some morphological and physiological changes which improves vigour.

Keywords: *Cucurbita maxima*, *Glomus geosporum*, *Mycorrhiza*, *Poultry manure*, *Salinity Stress*

1. INTRODUCTION

The presence of excessive soluble salts in soil such as Na⁺, Na⁺/K⁺, Mg²⁺/Ca²⁺ and Cl⁻/NO³⁻ diminishes nutrient and plant water uptake and interrupts the circulation of ions at both the cellular and the whole-plant levels, thereby prompting osmotic and ionic disparities [1]. When salts are present in the soil above certain precarious levels, this can result in numerous physiological and biological complications to plants [2]. First of all, the rate of photosynthesis is greatly affected by soil salinity stress [3]. Plants by increasing their photosynthetic rates are able to produce a higher biomass yield than when their photosynthetic rates are lower [3]; but, under soil salinity conditions, to conserve water, plants adapt by reducing the rate of opening of stomata and as a result reduce the rate of photosynthesis resulting in reduced carbon fixation by plants [3].

Conversely, one of the most essential salinity stress special effects on plants is caused by nutritional disarrays, which result from salinity effect on accessibility, availability, uptake and translocation of the nutrients within the plant [4]. Plant nutrient shortages as well as ionic toxicity and osmotic stress are dynamics accredited to the damaging effect of salinity on plant growth and yield [5]. Nevertheless,

salts can disrupt apoplastic conveyance between the cells owing to ion buildup and thwart water movement [3]. With respect to plant tissues, high salinity makes the tissues more inelastic and alters the cell wall elasticity [3]. Furthermore, soil salinization with the associated increase in plants' Na⁺ and Cl⁻ levels is supplementary with numerous internal difficulties due to inhibition of protein and enzyme synthesis [6]. Additionally, soil salinity damages the soil particles, plummeting porosity and the ability of plants to absorb water [6].

Recently, the increase demand for chicken meat has led to the rise in poultry farming resulting in the increase in the generation and utilization of organic wastes (e.g. chicken manure) as fertilizers. These organic wastes are very rich in organic matter, mineral nutrients and water [7]. The use of organic wastes such as poultry droppings as manure has been in practice globally for centuries now [8] and modern times [9]. However, the use of poultry droppings as manure and vastly overtaken the use other animal wastes such as cow dung, pig manure, kraal manure etc. because of the rich nitrogen, potassium and phosphorus content [10]. Also, the high price of inorganic fertilizers and their long term negative impacts on the environment has also encouraged the increased patronage of poultry manure. Recent studies have shown a host of nutrient management practices undertaken by smallholder African farmers in favour of poultry manure [11].

Several Investigations carried out to comprehend arbuscular mycorrhizal fungi (AMF)-salinity relations revealed that mycorrhizal fungi decrease undesirable effects of these stresses and stimulate plant growth and development [12, 13]. It has been extensively acknowledged that AMF increases water use efficiency and nutrient uptake of plant under salt stress conditions, hence facilitating the reduction of the damaging impression of salt stress [1]. AMF lessens the disadvantageous effects of toxic ions on membrane permeability and cell organelle, preserve the level of compatible organic solutes and intensifies antioxidant production (both enzymatic and non-enzymatic), and completely control expression of salt-related genes [1].

Cucurbita maxima belong to the family *Cucurbitaceae*. They have been reported from many countries in tropical Africa and probably occur in all countries. *C. maxima* are rarely found growing in the wild in Nigeria. It is cultivated in Northern Nigeria for the fruits. In southern Nigeria, in a largely unimproved form, it is cultivated for both the leaves and fruits [14]. 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 [15]. For venomous invertebrates' bites such as those of centipedes, the ripe gourd is cut, dried and made into a paste and applied to infected regions. The fresh seeds pulped or in emulsion, are used as antihelminthic [15].

Keeping abreast the beneficial effects of AMF and poultry manure there still exist a need to assess the potential impacts of chicken manure on soil chemical properties and its synergy with AMF (*Glomus geosporum*) on the yield and survival of *C. maxima* under soil salinity stress, hence, this research.

2. MATERIAL AND METHODS

2.1 Experimental Soil Sampling Site

The saline soil used for this study was 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 [16].

2.2 Experimental Setup

This experiment was set up in a complete block design with all treatments replicated thrice for *C. maxima*. This gave a total of four (4) treatments with three (3) replicates totaling twelve (12) combinations (Table 2.1).

Table 2.1: Experimental design

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

2.3 Experimental Soil Treatment and Planting

The experimental soil was steam sterilized in the oven in bits for two hours at 100°C to kill weed seeds and soil microorganisms and sieved through a 2 mm mesh to remove pebbles. The poultry manure was air dried for three (3) days before mixing about 4 kg of the manure to 10 kg of saline soil and left undisturbed for 1 week. AMF (*G. geosporum*) was inoculated by placing about 25 g of soil/root fragments containing about 60 – 65 spores per 5 g in planting hole at 15 cm depth, before planting the *C. maxima*. 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.

2.4 Physico-chemical Analysis of Experimental Soils

The experimental soil samples were analyzed following the standard procedures outlined by the Association of Official Analytical Chemist [17] procedure for wet acid digestions.

2.5 Determination of Growth Parameters

The shoot length, leaf area and nodes of healthy leaves from the experimental plants were taken 9 weeks after sprouting (WAS) using standard methods.

2.6 Determination of Photosynthetic Pigments

The atLeaf chlorophyll meter was used for non-destructive estimation of the total chlorophyll and separated into chlorophyll a, b and carotenoids contents of *C. maxima*.

2.7 Determination of Physiological Parameters

Electrolyte leakage was calculated using the formula EC1/EC2 as described by Shi *et al.* [18]. Values of FW, TW, and DW were used to calculate LRWC using the formula:

$$\text{LRWC (\%)} = \frac{(\text{FW}-\text{DW})}{\text{TW}-\text{DW}} \times 100 \quad \text{Kaya et al. [19]}$$

Plant vigor index in each treatment was calculated using the formula:

$$\text{Vigor index} = \text{Root length} + \text{Shoot length} \times \text{percentage emergence (\%)} \quad \text{Maisuria and Patel [20]}$$

Plant salt tolerance index (PSTI) was calculated using the formula of Jaarsma *et al.* [21]

$$\text{PSTI} = \frac{\text{Fresh weight salt treatment}}{\text{Fresh weight control}}$$

2.8 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 parameters analyzed. The differences between the means were separated and compared using the Duncan's multiple range tests. However, a probability level of $P = .05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

The physicochemical analysis of the experimental soils (saline and poultry manure augmented soils) indicated significant ($P = .05$) difference between the two soil types in parameters such as; pH, available phosphorus, total nitrogen, clay, sand, Ex. Ca, Mg, K, Na, OC and EC. Furthermore, no significant ($P = .05$) difference was observed in parameters such as; silt, Ex. Acidity, ECEC and base saturation between the two soil types (Table 3.1).

Table 3.1: Physicochemical properties of the experimental soils

S/No.	Parameters	Saline Soil Amended with Poultry Manure	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.	Exchangeable acidity (meq/100g)	3.52	3.22	-1.090
13.	ECEC (cmol./kg)	17.57	17.51	-3.401
14.	Base saturation (%)	79.97	81.61	-1.216
15.	EC. (dS/m)	0.29	6.70	-15.322*

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

The significant ($P = .05$) increase observed in EC, pH and Ex Na⁺ and the decrease in organic carbon, total nitrogen and available phosphorus in saline soil is similar to the results reported by Miller and

Gardiner [22], they reported an upsurge in pH and EC in saline soils in New Jersey due to soil salinity. Also, similar 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 were recorded by Deleke and Akomolafe [23].

The use of poultry manure to augment the adverse effect of soil salinity had significant ($P = .05$) alleviatory effect on the soil properties. Oagile and Namasiku [24] reported that the application of poultry manure showed great increase having exceptionally higher contents of exchangeable N, P and Ca as observed in this study.

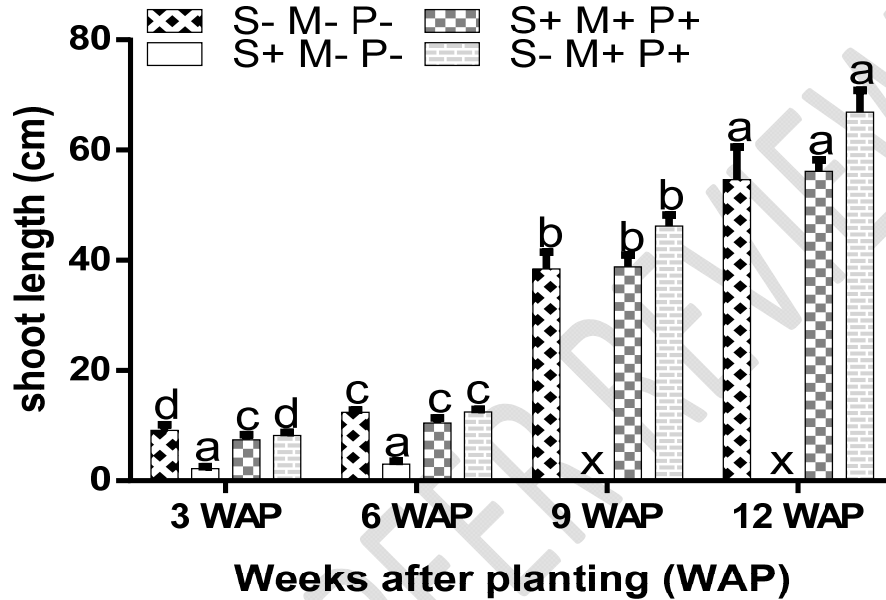


Figure 3.1. Influence of Poultry manure and AM Fungi synergy on shoot length of *C. maxima* grown in saline soil. Bars followed by same letter are not significantly different at $P = .05$ level. 'x' indicates that the plants died off.

Results obtained from this research showed that shoot length, number of leaves and leaf area of *C. maxima* were all significantly ($P = .05$) affected by soil salinity stress (Figure 3.1, 3.2 and 3.3). Several researchers corroborate this findings and reported on plants such as *Raphanus sativus* L. [25], *Vigna unguiculata* L. [26] and *Vigna mungo* L. [27]; They all reported that increased soil salinity results in a decline in the shoot lengths of the plants. NaCl has been revealed to reduce number of leaves in *Phaseolus acutifolius* L., *V. unguiculata* L. and *Phaseolus filiformis* L. [28]. Also, several researches has documented significant reduction in leaf area in *Vicia faba* [29], *Avena sativa* L. [30] and *Fragaria xananssa* L. [31]. This notable decrease in the growth parameters such as shoot length, number of leaves and leaf area in *C. maxima* planted under saline conditions in this study is as a result of the increased absorptions and accumulation of Na^+ and Cl^- . This could be explained by the undesirable influence of salt on photosynthetic processes and subsequently plant overall growth and leaf growth to avoid escape of water via transpiration and chlorophyll content [32].

Inoculation of *C. maxima* with arbuscular mycorrhizal fungi (AMF) (*Glomus geosporum*) and poultry manure (PM) synergy in combined form significantly ($P = .05$) increased the growth of *C. maxima* above the control in both saline and non-saline soil treatments. Similar observations have been made by Okon *et al.* [33] while working on AMF *Glomus deserticola* inoculation and mulch on *Manihot esculenta*. This can be attributed to the PM conserving moisture and nutrients, thus making the soil conditions conducive for the AMF colonization [33]. Similar observations have been made by Tanmay *et al.* [34] on *Oryza sativa*, Islam *et al.* [35] on hybrid rice.

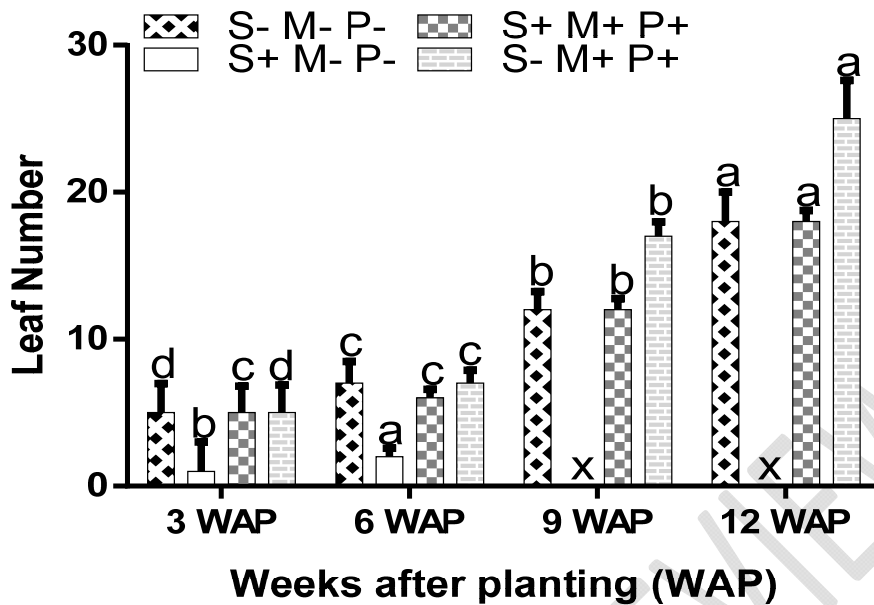


Figure 3.2. Influence of Poultry manure and AM Fungi synergy on leaf number of *C. maxima* grown in saline soil. Bars followed by same letter are not significantly different at $P = .05$ level. 'x' indicates that the plants died off.

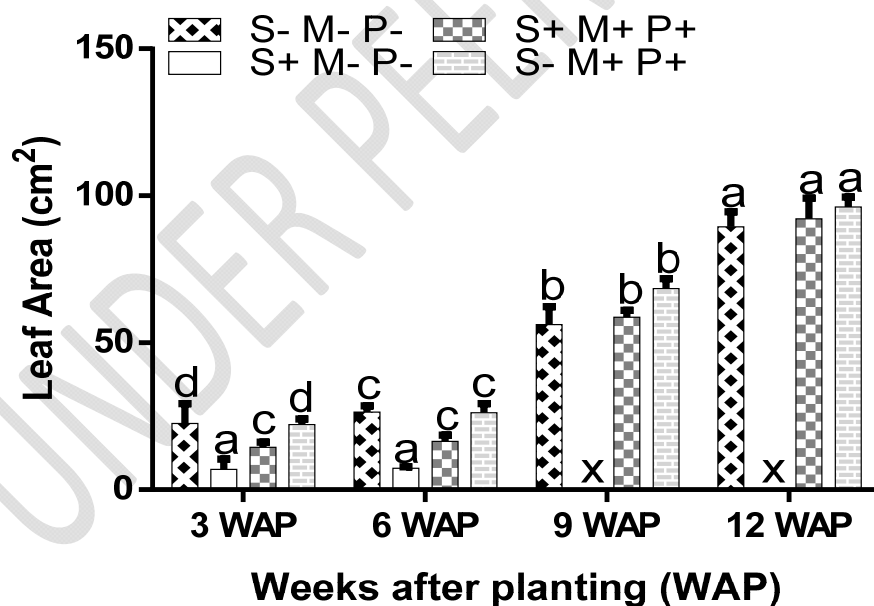


Figure 3.3. Influence of Poultry manure and AM Fungi synergy on leaf area of *C. maxima* grown in saline soil. Bars followed by same letter are not significantly different at $P = .05$ level. 'x' indicates that the plants died off.

Total photosynthetic pigments, chlorophyll a, b and carotenoids contents of *C. maxima* were significantly ($P = .05$) reduced by soil salinity (Figure 3.4). Non-mycorrhizal *C. maxima* treatment was more severely affected than the mycorrhizal inoculated plants. Jing *et al.* [36] reported significant

reduction in the total chlorophyll content of *Suaeda aralocaspica* exposed to high soil salinity, this reduction was ascribed to the decrease or destruction of the chloroplast structure. Wu *et al.* [12] reported that they observed increased photosynthetic rates and stomatal conductance in AMF over non-AMF plants under soil salinity stress. The increased rate of photosynthesis in AMF-colonized plants under salinity stress has been correlated with lower intercellular CO₂ concentration in mycorrhizal plants, since the higher photosynthetic capacity increases water use efficiency for the assimilation of more carbon per unit water transpiration [37].

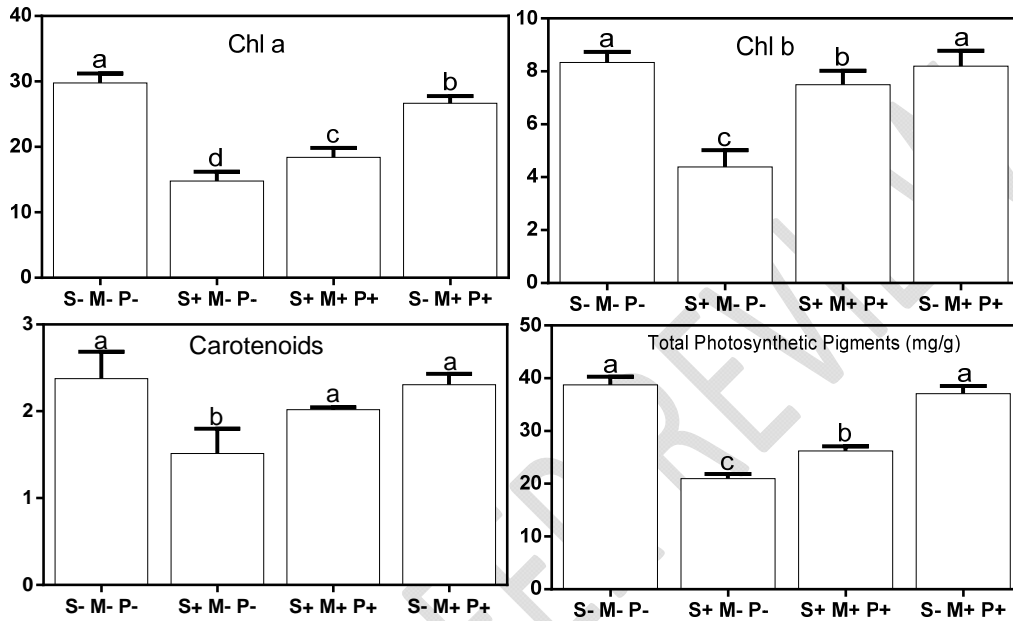


Figure 3.4. Influence of Poultry manure and AM Fungi synergy on chlorophyll a, b, c and total photosynthetic pigments of *C. maxima* grown in saline soil. Bars followed by same letter are not significantly different at $P = .05$ level.

C. maxima leaf turgid weight (LTW), leaf relative water content (LRWC), vigor index (VI) and salt tolerance index (STI) were significantly reduced by soil salinity, while electrolyte leakage (EL) was higher in saline soil treatment than non-saline soil treatments (Table 3.2). However, inoculation of *C. maxima* with AMF (*G. geosporum*) in conjunction with poultry manure amendment significantly ($P=0.05$) increased the LTW, LRWC, VI and STI in both saline and non-saline soil treatments. However, electrolyte leakage (EL) reduced with mycorrhizal inoculation (Table 3.2).

Ali and Hassan [38] reported that under salt stress because of inadequate water uptake, RWC was significantly decreased in relation to salinity in chamomile herb. Shou-Jun *et al.* [39] also reported that under no saline condition, leaf relative turgidities in non-mycorrhizal and mycorrhizal plants remained at comparatively steady-state level from 53.75% to 54.56% throughout the experiment. Mycorrhizal inoculation led to relatively higher leaf turgidity compared to non-mycorrhizal plants in this study. The phenomenon is ascribed to improved hydraulic conductivity of plants with a longer root and an altered root system morphology induced by AM fungi [39]. Sheng *et al.* [38] reported that plants inoculated with AMF maintain relatively higher water content compared with uninoculated plants. Inoculation with AMF often results in increased nutrient uptake, accumulation of an osmoregulator, an increase in photosynthetic rate and water use efficiency, suggesting that salt stress alleviation by AMF results from a combination of nutritional, biochemical and physiological effects [40].

Table 3.2: Impact of poultry manure and arbuscular mycorrhizal fungi (AMF) synergy on the physiological parameters of *C. maxima* grown in saline soil

Treatments	Leaf Turgid Weight (LTW) (g)	Leaf Relative Water Content (LRWC) (%)	Salt Tolerance Index (STI)	Vigor Index (VI) (%)	Electrolyte Leakage (EL) (dS/m)
S- M- P-	*1.67 ± 0.06 ^b	38.00 ± 2.51 ^b	1.00 ± 0.01 ^a	7481.00 ^b	0.81 ± 0.24 ^c
S+ M- P-	0.50 ± 0.10 ^c	11.30 ± 0.40 ^c	0.10 ± 0.03 ^c	150.00 ^d	3.04 ± 0.70 ^a
S+ M+ P+ (Gg)	1.26 ± 0.11 ^b	36.00 ± 1.24 ^b	0.36 ± 0.08 ^b	4303.00 ^c	2.02 ± 0.67 ^b
S- M+ P+ (Gg)	2.74 ± 0.61 ^a	45.21 ± 2.01 ^a	1.06 ± 0.54 ^a	10088.00 ^a	0.75 ± 0.44 ^c

*Mean of three replicates ± SEM. ^aMeans within of each column followed by different letters are significantly different at $P = .05$ according to Duncan's Multiple Range Test. (Gg) – *Glomus geosporum*, 0.00 (means the plants were dead).

Mumtaz *et al.* [41] reported that electrolyte leakage was enhanced with increasing salinity levels as compared to the control in salt sensitive cucumber plants as compared to the salt tolerant cultivar. This observation has been reported by other investigators in cucumber [42] and tomato [43]. A major effect of environmental stress (i.e., salt, drought) on plant is membrane modification, which results in cell membrane perturbed function or total dysfunction. Changes in membrane leakage and injury can be measured by the extent of EL (Electrolyte Leakage) in tissues [43]. The positive effects of AMF inoculation may result in improving integrity, vigour and stability of the membrane since the membrane permeability has been found to be reduced by AMF inoculation. Plants inoculated with AMF have been shown to maintain a lower electrolyte concentration than the non-mycorrhizal ones and hence maintain membrane stability [39].

4. CONCLUSION

Despite the complexity of the arbuscular mycorrhizal fungi, poultry manure and plant interaction in these experiments, the work presented in this research provides insights into the role of mycorrhiza (*G. geosporum*) treatments in conjunction with poultry manure in alleviating salt stress of some *C. maxima*. The findings of this research improves our understanding of the morphological and physiological responses of *C. maxima* to mycorrhizas in the context of salinity stress and shed more light on the importance of several factors, such as AMF type and enhancement by synergistic interaction between AMF and poultry manure, in determining the outcome of the fungus-plant relationship under salt stress to enhance plant growth and crop productivity, thus opening new horizons for future research.

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