Synergistic effect of arbuscular mycorrhizal fungi and poultry manure to significantly increase proximal structure and physiological parameters of *Cucurbita maxima* and *Telfairia occidentalis* under soil salinity

Okon G. Okon¹, Abdulnabi A. Matrood², Abdelhak Rhouma³,✉, Ukponobong E. Antia⁴

¹Department of Botany, Faculty of Biological Sciences, Akwa Ibom State University, Ikot Akpaden, Nigeria
²Department of Plant Protection, College of Agriculture, University of Basrah, Basrah, Iraq
³Higher Agronomic Institute of Chott Mariem Sousse, University of Sousse, Tunisia
⁴Department of Microbiology, Faculty of Biological Sciences, Akwa Ibom State University, Ikot Akpaden, Nigeria

✉Corresponding author: abdelhak.rhouma@gmail.com

**Abstract**

This research was carried out to assess the potential impacts of arbuscular mycorrhizal fungi (AMF) complex (*Glomus geosporum* and *Rhizophagus irregularis*) synergy with poultry manure (PM) on the survival of *Cucurbita maxima* and *Telfairia occidentalis* grown under salt stress conditions. This experiment was set up in a completely randomized design with all treatments replicated thrice for both test plants. Analysis of the saline and garden soils used in this study revealed significant (*P* ≤ 0.05) variations in their soil physico-chemical parameters. Increase in parameters such as pH (7.75 for saline soil; 6.78 for garden soil), EC (SS 7.80 dS.m⁻¹; GS 0.32) and Ex Na⁺ (SS 8.81 cmol.kg⁻¹; GS 0.4181 cmol.kg⁻¹) was observed in the saline soil while there was a decrease in organic carbon, total nitrogen and available phosphorus in saline soil. Proximate analysis of *C. maxima* and *T. occidentalis* leaves revealed that fiber, carbohydrate, and caloric value were slightly reduced in saline soil treatments while ash, protein and lipids contents were slightly increased. AMF inoculation and PM application had significant effect on proximate composition of these plant leaves but caloric value which was significantly (*P* ≤ 0.05) increased in non-saline soil treatments. Physiological parameters such as leaf turgid weight (LTW), leaf relative water content (LRWC), vigor index (VI), and salt tolerance index (STI) were significantly reduced by salinity, while electrolyte leakage (EL) was higher in saline soil treatments than non-saline soil treatments. However, inoculation with AMF in combination with PM amendment significantly increased LTW, LRWC, VI, and STI in both saline and non-saline soil treatments above single treatment with mycorrhizal species and poultry manure. However, EL reduced with mycorrhizal inoculation. The results of this work have shown that AMF and PM can enhance plants ability to tolerate salinity possibly through some morphological and physiological changes which improved water and nutrients uptake.

© University of SS. Cyril and Methodius in Trnava

**Introduction**

Soil salinity is a term used to describe the amount of mineral salts present in soil (Shan 2009). The mineral salts constitute a mixture of electrolytes. The major cations in saline soils include Na⁺, Ca²⁺, Mg²⁺, and K⁺. The major anions include Cl⁻, SO₄²⁻,
HCO\textsuperscript{3}, CO\textsubscript{3}\textsuperscript{2}, and NO\textsuperscript{3}. These constituents are usually reported in units of mg/L (ppm), mmol.L\textsuperscript{-1} or mmol charge.L\textsuperscript{-1} (meq.L\textsuperscript{-1}) in solution extracted from a soil saturated with water (Tanji 2002). Salinity is often measured as electrical conductivity (EC), a measure of the ability of a substance to conduct electricity. The natural factors contributing to salinization of soils include weathering of rocks, deposition of oceanic salts, topographical factor, groundwater table fluctuation, salt lake, scald, fallow period, and flood water. However, the major contributors to soil salinization are weathering of parental rocks, oceanic salt deposition, and groundwater table fluctuation. Rocks are mostly rich in sodium and other salts (Saxena et al. 2017). The most common man-made factors contributing to salinization are the irrigational water and the use of chemical fertilizers and pesticides. Irrigation is an important factor in the crop productivity. Therefore, the use of high salt-containing water for crop irrigation and poor management practices for appropriate leaching of salts lead to the accumulation of salts and deteriorate the crop fields (Zhu 2007).

Salinity not only decreases the agricultural production of most crops, but also, as a result of its effect on soil physicochemical properties, adversely affects the associated ecological balance of the area. The harmful impacts of salinity include: 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 (Hu and Schmidhalter 2002; Ashok et al. 2012). Crop species show a spectrum of responses to salt, although all have their growth, and eventually, their yield reduced by salt. Salt effects are the combined result of the complex interaction among different morphological, physiological, and biochemical processes (Ashok et al. 2012). Salinity may directly or indirectly inhibit cell division and enlargement and finally the growth of the whole plant. In addition to these factors, some other factors like water deficit (drought stress), ion toxicity, ion imbalance and soil compaction may cause growth reduction, injury of foliage, nutrient deficiencies, destruction of soil structure which ultimately hampers the growth of the plant. Some above ground visible morphological symptoms of plants are marginal yellowing/browning of foliage, premature fall of leaves, twig and branch die back, loss of vigor and stunted growth (Ashok et al. 2012).

The high concentration of salts in soil may induce three types of stresses: osmotic, ionic or oxidative, which drastically affect plant growth and productivity (Saxena et al. 2017). Osmotic stress leads to altered water potential, thereby reducing the water use efficiency of plant due to induction of physiological drought conditions (Saxena et al. 2017). Ionic stress causes disruption of ion homeostasis at both cellular and whole-plant levels, oxidative stress elicits release of reactive oxygen species, which inhibit cell growth and plant metabolism. Experiments carried out to understand AMF-salinity interaction revealed that mycorrhizal fungi reduce negative effects of these stresses and promote plant growth (Wu et al. 2006; Zuccarini and Okurowska 2008; Evelin et al. 2009; Wu et al. 2010a, 2010b).

Plants encounter a variety of biotic and abiotic stresses, which cause significant structural and functional changes that will result in decreased yield and vegetative traits (plant height, shoot length, number of branches, fresh and dry biomass, etc.) (Agha et al. 2021; Matrood and Rhouma 2021; Sofy et al. 2021a). In the other hand, salinity is among the most significant threats hindering global food security. Arbuscular mycorrhizal fungi and poultry manure (practice alone or in combination) were commonly used for agricultural production for their abilities to improve plant health to salinity stress (Solaiman et al. 2020; Zhang et al. 2020; Sallam et al. 2021; Sofy et al. 2021a, 2021b).

Arbuscular mycorrhizal (AM) fungi are among the most common soil fungi and the majority of plant species have associations with AM fungal species (Selvaraj and Chellappan 2006). It is thought that about 80 % of vascular plants form AM associations (Hodge 2000). Mycorrhizal fungi can help plants to survive and grow under different environmental conditions, and also help plants increase their reproductive output (Bolandnazar et al. 2007). The basic elements of the symbiosis are...
that the plant provides the mycorrhiza with carbohydrates while the fungi enhance the plant’s uptake of certain nutrients needed for growth (Selvaraj and Chellapan 2006). It has been estimated that in compensation for the additional nutrients and water provided by mycorrhizas, a plant must provide 20 % of its fixed carbon to the roots for mycorrhizal establishment and maintenance of the association (Ebrahim 2014). Little information is available about the influence of combination poultry manure/arthuscular mycorrhizal fungi on yield and quality of cucurbits. Elmer and Pignatello (2011) and Blackwell et al. (2015) pointed out that poultry manure application and arbuscular mycorrhizal fungi could serve as a sustainable nutrient alternative to chemical fertilizer. This is due to the potential of the fungi to utilize unavailable organic nutrients and increase the efficiency use of nutrients through improved nutrients uptake, which might enhanced plant growth and yield (Elmer and Pignatello 2011; Blackwell et al. 2015). Earlier researchers have clearly documented the positive effect of organic amendments in combination with arbuscular mycorrhizal fungi (Hammer et al. 2014, 2015) on plant growth and improve nutrients uptake (Abdullahi et al. 2015; Solaiman et al. 2019). Solaiman et al. (2020) pointed out that the cucumber growth responses to poultry manure application and arbuscular mycorrhizal symbiosis showed better plant growth and yield of cucumber and reduced the negative impact of stress salinity. This combination also improved the nutrient uptake and soil fertility of sandy soils (Mickan et al. 2016; Paymaneh et al. 2018; Solaiman et al. 2020). This research was undertaken to investigate the effect of salt stress on the proximate composition and physiological parameters of two different Cucurbits (Telfairia occidentalis and Cucurbita maxima) and determine ways of ameliorating its effect using arbuscular mycorrhizal species and poultry manure.

**Experimental**

*Study area*

Saline soil and salt water were collected from the saline ecosystem of Iwuochang, Ibieno Local Government Area (Latitude 4.56°N and Longitude 7.57°E), Akwa Ibom State, Nigeria, with an annual rainfall of about 4,021 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 4,000 mm and mean temperature variation of 26 – 36 °C (AKSG 2008). 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 Fig. 1.

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

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

*Experimental design*

This experiment was set up in a randomized complete block design and arranged in three blocks of 108 pots each for each species of cucurbits (C. maxima and T. occidentalis). This gave a total...
of 12 treatments (Table 1) for each species with nine replicates totaling 108 combinations for each species of cucurbits. This experiment was carried out in 1 March 2020 in a greenhouse.

Table 1. Experimental design.

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

Planting

The experimental soils were steam sterilized in the oven in bits for 2 h at 100 °C to kill weed seeds and soil microorganisms and sieved through a 2 mm mesh to remove pebbles. The poultry manure used in this research consists of moisture (39.69 %), N (2.47 %), P2O5 (2.84 %), K2O (1.84 %). Treatments consisted of 1 kg of poultry manure per pot. AM fungi Glomus geosporum and Rhizophagus irregularis (60 – 65 spores per 5 g) were purchased from International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. Matured seeds of Cucurbita maxima and Telfairia occidentalis were obtained from Akwa Ibom State Agricultural Development Project (AKADEP) in Etinan. The obtained seeds were selected to eliminate infected seeds. Five seeds each of C. maxima and T. occidentalis were sown in each of the pots filled with about 10 kg of sterilized soils. Arbuscular mycorrhiza fungi were inoculated by placing 25 g of soil/root fragments containing 60 – 65 spores per 5 g 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 15 d 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 roots treatment with saline water (100 mL) using an Erlenmeyer flask (250 mL) on the two cucurbits species was performed 1 d after the establishment of the mycorrhizal symbiosis and the treatments were spaced every 3 d until the end of the trial (30 June 2020). Assessments were conducted 90 d after the treatment (with poultry manure and AM Fungi). At the end of trial (30 June 2020), 27 plants (3 plants × 9 replicates) per treatment and per block were assessed for each cucurbit species (C. maxima and T. occidentalis).

Physico-chemical properties of experimental soils

Soil samples were taken using a 7-cm-diameter soil auger. A total of 108 samples per block were collected in sterile polythene bags. For each block, samples were mixed together into a single one. Nine soil samples (500 g) per block (3 blocks) and per replicate (3 replicates) were collected from each treatment after and before the trials and brought to the laboratory. 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 analysed for physico-chemical properties of the soil. Soil samples were analysed following the standard procedures outlined by the Association of Official Analytical Chemist (AOAC 2005) procedure for wet acid digestions (Rhouma et al. 2019). Soil physicochemical properties (pH, Total Nitrogen (%), Available Phosphorus (mg.kg⁻¹), Silt (%), Clay (%), Sand (%), Ex. Ca (cmol.kg⁻¹), Ex. Mg (cmol.kg⁻¹), Ex. Na. (cmol.kg⁻¹), Ex. K. (cmol.kg⁻¹),...
Organic Carbon (%), Exchangeable acidity (meq/100 g), ECEC (cmol.kg\(^{-1}\)), Base saturation (%) and Electrical conductivity (EC) (dS.m\(^{-1}\)) were determined for each samples (Rhouma et al. 2019).

**Analysis of water samples**

Nine water samples (1,000 mL) per replicate (3 replicates) were collected from saline (water used in treatment) and freshwater). A total of 27 samples were collected in sterile glass bottles (2,000 mL) and brought to the laboratory. Water pH, electrical conductivity, and total dissolved solid (TDS was measured using portable pH/EC/TDS/Temperature combined (HI 991301, Hanna Instruments Ltd., Leighton, UK). Dissolved oxygen was measured using digital portable analyzer JPB-607A Portable Dissolved Oxygen Analyzer (Tech Instrumentation Inc., Elizabeth, USA). Biological oxygen demand (BOD5), total alkalinity, acidity, chloride, calcium, nitrate, sulphate, phosphate, magnesium, sodium and potassium concentration was determined by the conventional method of the Association of Official Analytical Chemists (AOAC 2005). Salinity was determined using digital salt meter, which measures the salinity of seawater in parts per thousand (Rhouma et al. 2017).

**Determination of proximate content**

The determination of ash, lipid, carbohydrate and crude fibre was carried out using the standard methods described by Cella and Watson (2000) and Adeola et al. (2010). Caloric value was determined using a bomb calorimeter.

**Physiological parameters**

Electrolyte leakage was calculated using the formula (Shi et al. 2006): EC1/EC2. Values of FW, TW, and DW were used to calculate LRWC using the formula (Eq. 1; Kaya et al. 2003):

\[
LRWC (%) = \frac{(FW - DW)}{TW - DW} \times 100
\]

To determine the turgid weight (TW), leaves were soaked 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. The turgid weight of the leaves was determined by weighing the soaked leaves on a weighing balance and weight recorded (Kaya et al. 2003).

Plant vigor index in each treatment was calculated using the formula (Eq. 2; Maisuria and Patel 2009):

\[
\text{Vigor index} = \text{Root length} + \text{Shoot length} \times \text{percentage emergence (})\%
\]

Plant salt tolerance index (PSTI) was calculated using the formula of Jaarsma et al. (2013) (Eq. 3):

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

**Statistical analysis**

All data in the present study were subjected to analysis of variance (ANOVA) using Statistical package for Social Sciences (SPSS) and data are presented as standard error of mean (± SEM) of triplicate experiments. The student’s t-test was used to determine the significant difference between means of the soil and water parameters analyzed. The differences between the means were separated and compared using the Duncan’s multiple range tests. However, a probability level of \(P \leq 0.05\) was considered statistically significant.

**Results and Discussion**

Analysis of the saline and garden soils used in this study revealed significant variations in their soil physico-chemical parameters (\(P \leq 0.05\)). Significant increase in parameters such as pH (7.75), EC (7.80 dS.m\(^{-1}\)) and Ex Na\(^+\) (8.81 cmol.kg\(^{-1}\)) was observed in the saline soil while there was a decrease in organic carbon (1.61 %), total nitrogen (0.49 %) and available phosphorus (24.66 mg.kg\(^{-1}\)) in saline soil (\(P \leq 0.05\) (Table 2 and 3). This observation is in line with the work of Miller and Gardiner (2007) who reported an increase in pH and EC in saline soils in New Jersey due to salt stress. Deleke and Akomolafe (2013) 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 (Garg and Manchanda 2008). This might be responsible for the significant decrease in the soil OC, Total N and P observed in this work. High concentrations of salts in soil, especially Na⁺ ions drastically affect the basic structure of soil (Garg and Manchanda 2008). The presence of Na⁺ ions in the cation exchange complex makes the soil compact and subsequently decreases soil porosity and aeration, which hampers plant growth and hinders their productivity (Garg and Manchanda 2008). The presence of the salts of calcium and magnesium forms a white crust on the soil surface that changes soil water osmotic potential; therefore, plants growing in saline soils face salt-induced physiological drought conditions. Salinity impairs plant’s major processes such as photosynthesis, protein and lipid metabolism, nutrient acquisition, and ion homeostasis. Indeed, water moves out of the plant due to salt-induced osmotic stress, which makes the plant dehydrated and eventually leads to the death of the plant (Evelin et al. 2011).

Table 2. Physicochemical properties of the experimental soils.

| S/No. | Parameters                     | Soil before treatment with saline water | Soil after treatment with saline water |
|-------|---------------------------------|----------------------------------------|---------------------------------------|
| 1.    | pH                              | 6.78a                                  | 7.75b                                 |
| 2.    | Total Nitrogen [%]              | 2.27a                                  | 0.49b                                 |
| 3.    | Available P. [mg.kg⁻¹]          | 36.31⁴                                 | 24.66⁴                                |
| 4.    | Silt [%]                        | 4.00b                                  | 5.60b                                 |
| 5.    | Clay [%]                        | 4.20b                                  | 12.00b                                |
| 6.    | Sand [%]                        | 92.04b                                 | 82.40b                                |
| 7.    | Ex. Ca [cmol.kg⁻¹]              | 5.25b                                  | 2.97b                                 |
| 8.    | Ex. Mg [cmol.kg⁻¹]              | 4.36⁴                                  | 3.80⁴                                 |
| 9.    | Ex. Na [cmol.kg⁻¹]              | 0.41b                                  | 8.81⁴                                 |
| 10.   | Organic Carbon (%)              | 5.61b                                  | 1.61b                                 |
| 11.   | Exchangeable acidity (meq/100g) | 3.56⁴                                  | 3.20⁴                                 |
| 12.   | ECEC [cmol.kg⁻¹]                | 20.56⁴                                 | 20.26⁴                                |
| 13.   | Base saturation [%]             | 82.68b                                 | 84.20b                                |
| 14.   | EC. (dS.m⁻¹)                    | 0.32b                                  | 7.80b                                 |

* Significant at $P \leq 0.05$, Ex – Exchange, ECEC – Effective cation exchange capacity, EC – Electrical conductivity.

Table 3. Water analysis of the experimental irrigation water.

| S/No. | Parameters                     | Saline water (water used in treatment) | Fresh water |
|-------|---------------------------------|----------------------------------------|-------------|
| 1.    | pH                              | 7.70a                                  | 6.70b       |
| 2.    | EC [μS.cm⁻¹]                   | 3080.00⁴                               | 27.70b      |
| 3.    | TDS                            | 1021.00⁴                               | 11.00b      |
| 4.    | Acidity [mg.L⁻¹ as CaCo₃]       | 80.00⁴                                 | 95.40⁴      |
| 5.    | Alkalinity [mg.L⁻¹ as CaCo₃]    | 138.00⁴                                | 53.20⁴      |
| 6.    | DO [mg.L⁻¹]                    | 6.40b                                  | 7.60⁴       |
| 7.    | BOD [mg.L⁻¹]                   | 3.20b                                  | 2.80⁴       |
| 8.    | Sulphate [mg.L⁻¹]              | 102.31⁴                                | 1.91⁴       |
| 9.    | Phosphate [mg.L⁻¹]             | 0.09a                                  | 0.04a       |
| 10.   | Nitrate [mg.L⁻¹]               | 0.06b                                  | 2.82⁴       |
| 11.   | Cl⁻ [mg.L⁻¹]                   | 2560.13⁴                               | 55.23⁵      |
| 12.   | Ca²⁺ [mg.L⁻¹]                  | 55.71⁴                                 | 106.20⁴     |
| 13.   | Mg²⁺ [mg.L⁻¹]                  | 120.20⁴                                | 232.81⁴     |
| 14.   | Na⁺ [mg.L⁻¹]                   | 1027.00⁴                               | 0.11b       |
| 15.   | K⁺ [mg.L⁻¹]                    | 6.42b                                  | 8.40⁴       |
| 16.   | Salinity [ppt]                 | 33.21⁴                                 | 0.32b       |

* Significant at $P \leq 0.05$, EC – Electrical conductivity, DO – Dissolved oxygen, BOD – Biological oxygen demand, TDS – Total dissolved solids.
Proximate analysis of *C. maxima* and *T. occidentalis* leaves revealed thatibre, carbohydrate and caloric value were slightly reduced in saline soil treatments while ash, protein and lipids contents were slightly increased (Table 4 and 5). AMF inoculation of *C. maxima* and *T. occidentalis* and poultry manure application had some significant effect on proximate composition of these plant leaves but caloric value which was significantly increased in non-saline soil treatments ($P \leq 0.05$) (Table 4 and 5). Proximate composition of *C. maxima* and *T. occidentalis* such as carbohydrate, caloric value and fibre were significantly reduced with salinity, while ash, protein and lipids increased in saline soil treatments compared to non-saline soil treatments. However, inoculation of *C. maxima* and *T. occidentalis* with arbuscular mycorrhizal fungi (AMF) (*R. irregularis* and *G. geosporum*) in conjunction with soil amelioration with poultry manure (PM) increased some proximate composition of *C. maxima* and *T. occidentalis*. Proximate analysis of *C. maxima* and *T. occidentalis* leaves increased dramatically for the plants treated with *G. geosporum*+manure poultry (ash 18.48 – 17.09 %, respectively; fibre 15.20 – 20.11 %, respectively; protein 8.81 – 8.21 %, respectively; lipid 8.38 – 6.88 %, respectively; CHO 49.12 – 47.71 %, respectively; caloric value 297.60 – 281.41 Kcal, respectively) and *R. irregularis* + manure poultry (ash 17.54 – 17.02 %, respectively; fibre 14.67 – 19.82 %, respectively; protein 8.22 – 8.41 %, respectively; lipid 6.94 – 6.66 %, respectively; CHO 50.03 – 48.09 %, respectively; caloric value 318.71 – 289.00 Kcal, respectively) even in the presence of salinity (Table 4 and 5). It can be concluded from the results of this study that *C. maxima* plants tolerate better the salinity when treated with *G. geosporum*+manure poultry. We concluded that the addition of *R. irregularis* in combination with poultry manure to soil optimally improved the studied parameters of *T. occidentalis* in water stress conditions (Table 4 and 5).

Increase in ash, protein and lipids have also been reported by Uddin *et al.* (2017) in *Clinacanthus nutans* with increasing salinity. The findings in this study agree with the results of Ali *et al.* (2014) on *Portulaca oleracea*, *Hibiscus sabdariffa* and *Sorghum bicolor*, Kekere (2014) in *A. hypogea*, Uzun *et al.* (2013) and Kapoor and Srivastava (2010) who reported that increasing salinity levels tended to enhance crude protein synthesis. The increase in crude lipid content of *C. maxima* and *T. occidentalis* in response to salinity may be because plants have some level of tolerance at these salinity levels or due to accumulation of compatible solutes (Uddin *et al.* 2017). These results agree with those previously reported on *P. oleracea* by Teixeira and Carvalho (2009) and *Oenothera biennis* by Heuer *et al.* (2002). They observed increased total fat content in plants exposed to moderate saline environment.

**Table 4.** Effects of arbuscular mycorrhizal fungi (AMF) inoculation on the proximate composition of *C. maxima* grown in saline soil and ameliorated with poultry manure.

| Treatments | Ash [%] | Fibre [%] | Protein [%] | Lipid [%] | CHO [%] | Caloric value [Kcal] |
|------------|---------|-----------|-------------|-----------|--------|---------------------|
| S- M- P-   | 16.06 ± 0.66^a | 16.11 ± 0.38^a | 8.95 ± 0.21^a | 6.54 ± 0.15^a | 52.33 ± 4.55^a | 314.73 ± 10.51^a |
| S+ M- P-   | 0.00 ± 0.00^b | 0.00 ± 0.00^b | 0.00 ± 0.00^b | 0.00 ± 0.00^b | 0.00 ± 0.00^b | 0.00 ± 0.00^b |
| S+ M+ P- (Gg) | 18.65 ± 1.24^a | 16.21 ± 0.33^a | 10.71 ± 1.61^a | 8.22 ± 0.25^a | 46.21 ± 3.42^b | 242.71 ± 8.41^f |
| S+ M+ P- (Ri) | 18.40 ± 0.71^a | 15.72 ± 0.61^a | 9.20 ± 0.75^a | 8.61 ± 0.34^a | 48.07 ± 3.11^b | 257.10 ± 8.11^e |
| S+ M+ P+ (Gg) | 19.06 ± 1.62^a | 15.68 ± 0.46^a | 11.24 ± 1.22^a | 8.68 ± 0.29^a | 45.34 ± 2.05^b | 239.12 ± 6.34^f |
| S+ M+ P+ (Ri) | 18.48 ± 1.44^a | 15.20 ± 0.51^a | 8.81 ± 0.61^b | 8.38 ± 0.41^c | 49.12 ± 4.12^a | 279.60 ± 6.81^d |
| S+ M+ P+ (Ri) | 17.54 ± 1.34^a | 14.67 ± 0.25^a | 8.22 ± 0.57^b | 6.94 ± 0.38^c | 50.03 ± 4.48^a | 318.71 ± 9.66^c |
| S+ M+ P+ (Gg) | 16.76 ± 0.84^b | 15.49 ± 0.35^a | 7.97 ± 0.43^b | 6.61 ± 0.31^b | 53.17 ± 4.33^a | 320.12 ± 10.67^c |
| S+ M+ P+ (Ri) | 16.24 ± 0.69^b | 16.01 ± 0.42^b | 7.77 ± 0.64^b | 6.58 ± 0.50^b | 53.40 ± 4.37^a | 322.50 ± 10.81^d |
| S+ M+ P+ (Gg) | 15.45 ± 0.51^c | 14.81 ± 0.29^b | 8.96 ± 0.72^b | 6.62 ± 0.34^c | 54.16 ± 4.71^a | 332.13 ± 12.12^c |
| S+ M+ P+ (Ri) | 16.28 ± 0.75^c | 14.77 ± 0.32^b | 8.06 ± 0.76^b | 6.48 ± 0.34^c | 54.41 ± 4.21^a | 351.00 ± 14.32^c |
| S- M+ P-   | 17.69 ± 0.65^d | 14.58 ± 0.45^a | 8.09 ± 0.59^b | 7.92 ± 0.51^b | 51.02 ± 3.55^a | 298.30 ± 9.42^d |

*Mean of three replicates ± SEM. Means within 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*, 0.00 (means the plants were dead).*
Measured physiological parameters of *C. maxima* and *T. occidentalis* such as leaf turgid weight (LTW), leaf relative water content (LRWC), salt tolerance index (STI) and vigor index (VI) were significantly reduced in saline soil treatments when compared to the control, while electrolyte leakage increased in saline soil treatments (*P* < 0.05) (Table 6 and 7). Amelioration of the saline soil with poultry manure alone also enhanced the physiological parameters of *C. maxima* and *T. occidentalis*. Inoculation with AMF alone or together with poultry manure amelioration significantly increased performance of the physiological parameters in the two test plants both in saline and non-saline soil treatments during the first and second cropping seasons (*P* ≤ 0.05) (Table 6 and 7).

LTW, LRWC, STI and VI were all significantly higher in AMF inoculated and poultry manure ameliorated *C. maxima* plants grown in non-saline soil (*P* ≤ 0.05) (Table 6 and 7).

### Table 5. Effect of arbuscular mycorrhizal fungi (AMF) inoculation on the proximate composition of *T. occidentalis* grown in saline soil and ameliorated with poultry manure.

| Treatments | Ash [%] | Fibre [%] | Protein [%] | Lipid [%] | CHO [%] | Caloric value [Kcal] |
|------------|---------|-----------|-------------|-----------|---------|---------------------|
| S- M- P-   | *17.20 ± 1.51* | 20.17 ± 2.78 | 8.74 ± 0.15 | 6.81 ± 0.57 | 47.08 ± 3.58 | 298.16 ± 6.45 |
| S+ M+ P-   | 21.43 ± 1.91 | 23.11 ± 2.88 | 8.67 ± 0.31 | 8.27 ± 0.66 | 38.52 ± 2.94 | 197.14 ± 4.88 |
| S+ M+ P- (Gg) | 18.96 ± 0.52 | 21.19 ± 1.94 | 8.21 ± 0.25 | 7.08 ± 0.46 | 45.45 ± 3.42 | 246.30 ± 6.12 |
| S+ M+ P- (Ri) | 17.47 ± 0.78 | 21.43 ± 1.81 | 8.87 ± 0.38 | 6.71 ± 0.49 | 45.52 ± 3.64 | 275.28 ± 5.14 |
| S+ M+ P+   | 22.00 ± 1.24 | 21.77 ± 1.57 | 8.77 ± 0.42 | 8.25 ± 0.67 | 39.21 ± 3.11 | 202.11 ± 3.88 |
| S+ M+ P+ (Gg) | 17.09 ± 0.64 | 20.11 ± 1.45 | 8.21 ± 0.33 | 6.88 ± 0.35 | 47.71 ± 3.31 | 281.41 ± 5.74 |
| S+ M+ P+ (Ri) | 17.02 ± 0.45 | 19.82 ± 1.39 | 8.41 ± 0.47 | 6.66 ± 0.42 | 48.09 ± 4.01 | 289.00 ± 6.14 |
| S- M+ P- (Gg) | 16.42 ± 0.64 | 18.76 ± 1.43 | 7.91 ± 0.51 | 6.21 ± 0.29 | 50.70 ± 3.69 | 301.10 ± 7.22 |
| S- M+ P- (Ri) | 16.53 ± 0.51 | 18.64 ± 0.84 | 7.61 ± 0.39 | 6.11 ± 0.43 | 51.11 ± 4.12 | 307.42 ± 7.31 |
| S- M+ P+ (Gg) | 17.06 ± 0.48 | 18.65 ± 1.44 | 7.66 ± 0.45 | 6.06 ± 0.28 | 51.57 ± 4.24 | 309.40 ± 6.48 |
| S- M+ P+ (Ri) | 16.22 ± 0.33 | 18.70 ± 0.87 | 7.25 ± 0.42 | 6.21 ± 0.57 | 51.62 ± 4.1 | 311.10 ± 7.24 |
| S- M- P+   | 17.03 ± 0.66 | 19.76 ± 1.46 | 7.84 ± 0.37 | 5.29 ± 0.61 | 50.08 ± 3.82 | 299.90 ± 6.75 |

*Mean of three replicates ± SEM. aMeans 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*, 0.00 (means the plants were dead).*

### Table 6. Effects of arbuscular mycorrhizal fungi (AMF) on the physiological parameters of *C. maxima* grown in saline soil ameliorated with poultry manure.

| Treatments | Leaf Turgid Weight (LTW) [g] | Leaf Relative Water Content (LRWC) [%] | Salt Tolerance Index [STI] | Vigour Index (VI) | Electrolyte Leakage (EL) [dS.m⁻¹] |
|------------|-----------------------------|------------------------------------|--------------------------|--------------------|-------------------------------|
| S- M- P-   | *0.67 ± 0.06*               | 38.00 ± 2.51                       | 1.00 ± 0.01              | 7481.00 ± 7.04    | 0.81 ± 0.24                   |
| S+ M+ P-   | 0.00 ± 0.00                  | 0.00 ± 0.00                        | 0.00 ± 0.00              | 0.00 ± 0.00       | 0.00 ± 0.00                   |
| S+ M+ P- (Gg) | 0.26 ± 0.11                | 36.00 ± 1.24                       | 0.36 ± 0.08              | 1,303.00 ± 6.06   | 2.22 ± 0.67                   |
| S+ M+ P- (Ri) | 0.23 ± 0.20                | 36.36 ± 1.66                       | 0.29 ± 0.06              | 956.00 ± 8.13     | 2.31 ± 0.38                   |
| S+ M+ P+   | 0.21 ± 0.09                  | 30.00 ± 1.02                       | 0.28 ± 0.12              | 856.00 ± 4.90     | 2.52 ± 0.44                   |
| S+ M+ P+ (Gg) | 0.68 ± 0.12                | 44.78 ± 2.42                       | 0.71 ± 0.33              | 8,037.00 ± 9.02   | 1.66 ± 0.14                   |
| S+ M+ P+ (Ri) | 0.69 ± 0.34                | 42.65 ± 1.57                       | 0.71 ± 0.24              | 7,897.00 ± 8.37   | 1.51 ± 0.16                   |
| S- M+ P- (Gg) | 0.70 ± 0.30                | 44.93 ± 3.10                       | 1.04 ± 0.67              | 9,465.00 ± 9.53   | 0.72 ± 0.20                   |
| S- M+ P- (Ri) | 0.69 ± 0.27                | 45.59 ± 1.67                       | 1.03 ± 0.75              | 8,378.00 ± 8.86   | 0.91 ± 0.48                   |
| S- M+ P+ (Gg) | 0.74 ± 0.61                | 45.21 ± 2.01                       | 1.06 ± 0.54              | 10,088.00 ± 10.75 | 0.85 ± 0.44                   |
| S- M+ P+ (Ri) | 0.93 ± 0.84                | 51.09 ± 3.44                       | 1.14 ± 0.68              | 10,583.00 ± 12.15 | 0.67 ± 0.28                   |
| S- M- P+   | 0.68 ± 0.22                  | 44.78 ± 1.97                       | 1.01 ± 0.24              | 8,099.00 ± 7.14   | 0.83 ± 0.41                   |

*Mean of three replicates ± SEM. aMeans 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*, 0.00 (means the plants were dead).*
Table 7. Effects of arbuscular mycorrhizal fungi (AMF) on the physiological parameters of *T. occidentalis* grown in saline soil ameliorated with poultry manure.

| Treatments | Leaf Turgid Weight (LTW) kg | Leaf Relative Water Content (LRWC) % | Salt Tolerance Index (STI) | Vigour Index (VI) | Electrolyte Leakage (EL) dS.m⁻¹ |
|------------|-----------------------------|-------------------------------------|---------------------------|------------------|-----------------------------|
| S- M- P-   | *0.89 ± 0.51a*              | *56.58 ± 3.11b*                    | *1.00 ± 0.01c*            | *11,984.00 ± 3.68* | *0.51 ± 0.24c*              |
| S+ M- P-   | *0.51 ± 0.20a*              | *37.82 ± 1.05d*                    | *0.28 ± 0.08g*            | *662.00 ± 3.80b*   | *2.06 ± 0.97a*              |
| S+ M+ P- (Gg) | *0.66 ± 0.32b*            | *49.09 ± 1.57c*                    | *0.48 ± 0.10f*            | *8,784.00 ± 2.70i* | *1.91 ± 0.11b*              |
| S+ M+ P- (Ri) | *0.71 ± 0.55b*           | *47.37 ± 1.22e*                    | *0.50 ± 0.25f*            | *9,265.00 ± 6.00b* | *1.76 ± 0.21b*              |
| S+ M+ P+   | *0.63 ± 0.19b*              | *52.73 ± 1.67b*                    | *0.61 ± 0.33e*            | *8,265.00 ± 4.33j* | *2.01 ± 0.40a*              |
| S+ M+ P+ (Gg) | *0.83 ± 0.22a*            | *57.75 ± 2.15b*                    | *1.02 ± 0.66c*            | *10,602.00 ± 7.09d* | *1.27 ± 0.22c*              |
| S+ M+ P+ (Ri) | *0.85 ± 0.46a*           | *60.27 ± 1.97a*                    | *1.21 ± 0.58b*            | *12,169.00 ± 2.64c* | *1.14 ± 0.31b*              |
| S- M+ P- (Gg) | *0.88 ± 0.75a*           | *63.38 ± 2.28a*                    | *1.23 ± 0.64b*            | *13,248.00 ± 4.64a* | *0.47 ± 0.26c*              |
| S- M+ P- (Ri) | *0.91 ± 0.69a*           | *66.67 ± 2.11a*                    | *1.37 ± 0.69a*            | *13,665.00 ± 1.95c* | *0.49 ± 0.34c*              |
| S- M+ P+ (Gg) | *0.95 ± 0.56a*           | *68.49 ± 3.15e*                    | *1.40 ± 0.81a*            | *13,901.00 ± 2.84b* | *0.52 ± 0.58c*              |
| S- M+ P+ (Ri) | *0.99 ± 0.87a*           | *70.67 ± 3.47a*                    | *1.07 ± 0.55c*            | *14,183.00 ± 8.28a* | *0.48 ± 0.61c*              |
| S- M- P+   | *0.89 ± 0.61a*              | *57.89 ± 2.30b*                    | *1.00 ± 0.01c*            | *12,201.00 ± 7.38j* | *0.53 ± 0.42c*              |

*Means with three replicates. *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*, 0.00 (means the plants were dead).

EL was however, highest in sole poultry manure ameliorated saline soil treatments. A similar trend was observed in *T. occidentalis*, except for EL which was highest in pure saline soil non-fertilized with poultry manure and uninoculated with AMF in the first cropping (Table 6 and 7).

The symbiotic association of *G. geosporum*-manure poultry applied on *C. maxima* and *T. occidentalis* plants allows better leaf turgid weight (0.68 – 0.83 g, respectively), leaf relative water content (44.78 – 57.75 %, respectively), salt tolerance index (0.71 – 0.88, respectively), vigour index (8,037 – 10,602 %, respectively), and electrolyte leakage (1.66 – 1.27 ds.m⁻¹, respectively) underwater stress conditions; whose *T. occidentalis* plants are more tolerant to water salinity. Also, the highest value of leaf turgid weight (0.69 – 0.85 g, respectively), leaf relative water content (42.65 – 60.27 %, respectively), salt tolerance index (0.71 – 1.02, respectively), vigour index (7,897 – 12,169 %, respectively), and electrolyte leakage (1.51 – 1.14 ds.m⁻¹, respectively) was obtained when the *C. maxima* and *T. occidentalis* plants are treated with *R. irregularis*-manure poultry under water stress conditions; whose *T. occidentalis* plants are more tolerant to water salinity (Table 6 and 7).

Foliar Na⁺ accumulation in of *C. maxima* and *T. occidentalis* had corresponding significant reduction on some physiological parameters such as leaf turgid weight (LTW), leaf relative water content (LRWC), salt tolerance index (STI), vigour index (VI) and Electrolyte leakage (EL) of *C. maxima* and *T. occidentalis* (*P ≤ 0.05*) (Fig. 2 and 3). Amelioration with poultry manure showed some improvements in these physiological parameters in the two test plants. Inoculation of *C. maxima* and *T. occidentalis* with AMF in non-saline soil treatments showed significant increase in physiological parameters when compared to the control and saline treatments (*P ≤ 0.05*) (Fig. 2 and 3). LTW, LRWC, STI, VI and EL were improved when the plants were treated with *G. geosporum*-manure poultry and *R. irregularis*-manure poultry underwater stress conditions. We can conclude that these treatments enhanced the studied parameters of *T. occidentalis* in water stress conditions (Fig. 2 and 3).

Physiological parameters of *C. maxima* and *T. occidentalis* such as leaf turgid weight (LTW), leaf relative water content (LRWC), vigour index (VI) and salt tolerance index (STI) were significantly reduced by salinity, while electrolyte leakage (EL) was higher in saline soil treatments than non-saline soil treatments. However, inoculation of *C. maxima* and *T. occidentalis* with AMF (*R. irregularis* and *G. geosporum*) in conjunction with poultry manure amendment significantly increased their leaf turgid weight.
(LTW), leaf relative water content (LRWC), vigor index (VI) and salt tolerance index (STI) in both saline and non-saline soil treatments above single treatment with mycorrhizal species and poultry manure ($P \leq 0.05$). However, electrolyte leakage (EL) reduced with mycorrhizal inoculation.

Fig. 2. Comparative assessment of the influence of foliar Na$^+$ accumulation on leaf turgid weight, leaf relative water content, salt tolerance index, vigour index and electrolyte leakage of *C. maxima*.
Fig. 3. Comparative assessment of the influence of foliar Na\textsuperscript{+} accumulation on leaf turgid weight, leaf relative water content, salt tolerance index, vigour index and electrolyte leakage of *T. occidentalis*.

The increasing of the studied parameters in the cucurbit plants with application of arbuscular mycorrhizal fungi and poultry manure could be attributed to two processes: (i) through increased
nutrient transport found in the poultry manure through the arbuscular mycorrhizal fungi and (ii) direct connection between cucurbits plant and mycorrhizal hyphal network. Arbuscular mycorrhizal fungi and poultry manure application are widely claimed to be able to aid plants under soil salinity conditions. Cyril et al. (2014), and Adebiyi and Adewole (2019) showed that organic manures particularly poultry manure, mixed with arbuscular mycorrhizal fungi had positive effects in increasing red amaranth yield and improving crop quality under saline conditions. Abusuwar (2018) reported that the arbuscular mycorrhizal fungi coupled with poultry manure enhanced leaf area, number of leaves per plant, plant height, fresh and dry biomass, yield and nutritive value of forage crops grown in saline soils and irrigated with saline water. Mickan et al. (2016) poultry manure applied to the agricultural soils with the presence of AM fungi stimulated growth of extra-radical hyphae in soil and increased mycorrhizal colonization of roots. Based on the understanding of these interactions, it has been claimed that AM fungi can be more important to plant growth and yield than poultry manure (Mickan et al. 2016; Solaiman et al. 2019, 2020; Zhang et al. 2020).

Over the last couple of decades, the universal symbiosis between arbuscular mycorrhizal fungi and cucurbits is such an old tie that, perhaps, enabled the establishment of plants in land (Rouphael et al. 2015; Chen et al. 2017) by boosting photosynthesis, plant growth, nutrient acquisition and decreasing membrane leakage and reactive oxygen species under condition of salinity stress (Cavagnaro et al. 2015; Liu et al. 2016; Sofy et al. 2021a). This symbiosis had been reported 400 million years ago (Selosse et al. 2015). Several research studies have reported the efficiency of AM fungi to impart growth and yield enhancement in plants under salinity stress (Abdel Latef and Chaoxing 2014; Talaat and Shawkly 2014). Wang et al. (2018) have noted considerable improvement in fresh and dry biomass, and N concentration of root and shoot due to mycorrhizal inoculation under saline conditions. Chen et al. (2017) revealed that the stem diameter, plant height, dry weight, root to shoot ratio of cucumber seedlings inoculated with Glomus sp., and Rhizophagus sp. were improved greatly compared with the non-inoculated control. The same authors showed that Glomus sp., and Rhizophagus sp. increase the nutrient concentration, net photosynthetic rate, chlorophyll content, root activity, light saturated rate of the CO2 assimilation, maximum carboxylation rate and maximum ribulose-1,5-bis-phosphate regeneration rate. Moreover, arbuscular mycorrhizal fungi can significantly improve plant nutrient uptake and resistance to several abiotic stress factors particularity salinity stress (Sun et al. 2018; Begum et al. 2019). Liu et al. (2016) documented that the AM fungi inoculation could effectively enhance the cucumber growth and other cucurbit crops, which is closely associated with the secondary metabolism in plants. Notably, Yang et al. (2014) and He et al. (2017) showed a positive stimulatory effect of Glomus spp., and Rhizophagus spp. on peanut and tomato in terms of photosynthetic characteristics, growth and hormone status. Yadav et al. (2013) found that the inoculation of Gloriosa superba with Glomus sp. can interact synergistically and maximize benefits, resulting in root length, higher leaf area and colchicine content. It is widely believed that arbuscular mycorrhizal fungi have been considered as an alternative to inorganic fertilizers in the near future (Ortas 2012), which is probably why AM fungi enhanced plant tolerance to abiotic factors (Plussard and Dell 2010). Qiu et al. (2019) documented that the soil application of arbuscular mycorrhizal fungi could improve the chemical and biological properties of soil and enhance their nutrient levels and enzymatic activities. A prominent role of such AM fungi application is to transfer nutrients, (organic carbon in the form of sugars and lipids) (Jiang et al. 2017; Lugimbuehl et al. 2017). Amiri et al. (2017) revealed that AM fungi-Pelargonium graveolens symbiosis increased the concentrations of N, P, and Fe under drought stress. Gomez-Bellot et al. (2015) reported the levels of P, Ca, and K in Euonymus japonica have been enhanced under salinity stress due to instant AM fungi attachment. In addition, AM fungi application improved P and N contents in plant tissues of Chrysanthemum morifolium (Wang et al. 2018) and increased seedling biomass by enhancing intercellular CO₂, P, and N contents and water content in Leymus chinensis (Jixiang et al. 2017). Furthermore, Hashem et al. (2018) documented that the synthesis of jasmonic acid,
salicylic acid, and several important inorganic nutrients and the total concentrations of P, Ca²⁺, N, Mg²⁺, and K⁺ were higher in the arbuscular mycorrhizal fungi-treated Cucumis sativus plants compared with those in the un-inoculated plants under salt stress conditions. Ali and Hassan (2014) 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. (2014) 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 (Shou-Jun et al. 2014). Sheng et al. (2008) 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 osmo-regulator, 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 (Evelin et al. 2009). Sofy et al. (2021a) proved that the combination of arbuscular mycorrhizal and organic amendment is the most effective in decreasing the damaging impacts of salt on spinach plants by increasing the up-regulation of antioxidants, morphological and physiological parameters (shoot and root length, fresh and dry biomass, membrane stability index, relative water content, mineral contents, chlorophyll content, total soluble protein content and endogenous phytohormones (auxin, abscisic acid, gibberellins, salicylic acid and jasmonic acid)) and decreasing membrane leakage and reactive oxygen species. Mumtaz et al. (2013) 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 (Kaya et al. 2001), rice (Lutts et al. 1996), tomato (Atilla 2014) and sugar beet (Ghoulam et al. 2002). Agha et al. (2021) and Sofy et al. (2021b) documented that the bacteria combination was the most efficient in reducing the harmful effects of salt on soybean and pea plants by boosting antioxidant up-regulation and lowering membrane leakage and reactive oxygen species.

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 (Atilla 2014). The positive effects of AM fungi inoculation may result in improving integrity, vigour and stability of the membrane since the membrane permeability has been found to be reduced by AM fungi inoculation. Plants inoculated with AMF have been shown to maintain a lower electrolyte concentration than the non-mycorrhizal ones and hence maintain membrane stability (Garg and Manchanda 2008; Ali and Hassan 2014).

Poultry manure has been widely used as fertilizers for centuries. This manure contains not only the basic nutrients required by crops, but also trace elements. It is rich in mineral elements, essential nutrients N, P, and K, and other nutrients that can stimulate microbial activity and healthy plants, ameliorate seed germination, increase vegetative traits, reduce the negative impact of salinity, increase the percent root colonization of arbuscular mycorrhizal fungi, improve the physical and chemical properties of the soil (structure, texture, porosity, etc.), maintain the balance of soil nutrient, improve nutrient absorption, increase root vitality, increase fertilizer retention, enhance yields, and participate in the addition of organic matter to organically-deficient soils (Mufwanzala and Dikinya 2010; Revell et al. 2012; Awad 2016; Pandian et al. 2016; Sikder et al. 2019; Sistani et al. 2019; Solaiman et al. 2020; Zhang et al. 2020; Sallam et al. 2021). Hirzel et al. (2018) showed that the highest values of available P and exchangeable K, S, Ca and Mg were obtained using poultry manure in saline soil, whereas pH and salinity (electrical conductivity) reported the lowest values. Adeleye et al. (2010) documented that poultry manure application enhanced soil physico-chemical properties by decreasing soil bulk density and temperature, and increasing exchangeable Ca,
Mg, K, total porosity, soil moisture retention capacity, total N, soil organic matter, available P and lowered exchange acidity under salinity conditions.

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 growth parameters, proximate composition and physiological parameters of C. maxima and T. occidentalis. The effects of mycorrhizal symbiotic association on C. maxima and T. occidentalis showed improvements on the growth of the test plants. Using different mechanisms C. maxima and T. occidentalis by itself or in association with arbuscular mycorrhizal fungi and poultry manure can tolerate or survive soil salinity. However, in the presence of the fungi, plant ability to resist the stress increases as a result of morphological and physiological changes and improved vigour, 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. T. occidentalis showed better salt tolerance indices on individual parameters and overall salt tolerance index. Given the importance of these strategies, arbuscular mycorrhizal fungi/poultry manure application has important implications under pot experiments. Hence, field and greenhouse trials aimed at understanding the effectiveness, efficiency and durability of these strategies are suggested for future studies.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Abdulahi R, Lihan S, Edward R, Demie LS (2015) Effect of arbuscular mycorrhizal fungi and poultry manure on growth and nutrients contents of maize in different soil type. J. Adv. Agric. 4: 428-437.

Abusuwar AO (2018) Effect of arbuscular mycorrhizal fungi (AMF) and fermented organic manure on yield and quality of forage sorghum (Sorghum bicolor L. Moench) var. Panar under saline conditions. J. Agric. Res. 3: 1-10.

Adebisi EA, Adewole MB (2019) Effects of arbuscular mycorrhiza fungi, organic fertilizer and different moisture regimes on soil properties and yield of Amaranthus cruentus. J. Agric. Sci. 64: 147-163.

Adeleye EO, Ayeni LS, Ojeniyi SO (2010) Effect of poultry manure on soil physico-chemical properties, leaf nutrient contents and yield of yam (Dioscorea rotundata) on Alfisol in Southwestern Nigeria. Am. J. Sci. 6: 871-878.

Adeola YB, Augusto CO, Adepoju OT (2010) Proximate and mineral composition of whole and dehulled nigerian sesame seed. Afr. J. Food Sci. 1: 71-75.

Agha MS, Abbas MA, Sofy MR, Haroun SA, Mowafy AM (2021) Dual inoculation of Bradyrhizobium and Enterobacter alleviates the adverse effect of salinity on Glycine max seedling. Not. Bot. Horti. Agrobot. Cluj. Napoca 49: 12461.

AKSG (Akwa Ibom State Government) (2008) Geography and location about Akwa Ibom State.

Ali AS, Mohamed BF, Dreyling G (2014) Salt tolerance and effects of salinity on some agricultural crops in the Sudan. For. Prod. J. 3: 56-65.

Ali EF, Hassan FAS (2014) Alleviatory effects of salt stress by mycorrhizal fungi and gibberellic acid on chamomile plant. Int. J. Sci. Res. 3: 109-111.

Amiri R, Ali N, Nematollah E, Mohammad RS (2017) Nutritional status, essential oil changes and water-use efficiency of rose geranium in response to arbuscular mycorrhizal fungi and water deficiency stress. Symbiosis 73: 15-25.

AOAC (2005) Official methods of analysis. 10th and 17th Ed., Association of Official Analytical Chemists, Washington D. C.

Ashok A, Nisha K, Karishma N, Anju T, Gupta KK (2012) Arbuscular mycorrhizal symbiosis and alleviation of salinity stress. J. Appl. Nat. Sci. 4: 144-155.

Atilla LT (2014) Influence of foliarly applied different triazole compounds on growth, nutrition, and antioxidant enzyme activities in tomato (Solanum lycopersicum L.) under salt stress. Aust. J. Crop Sci. 8: 71-79.

Awad MYM (2016) Poultry manure and humic acid foliar applications impact on caraway plants grown on a clay loam. J. Soil Sci. Agric. Eng. Mansoura Univ. 7: 1-10.

Begum N, Qin C, Ahanger MA, Raza S, Khan MI, Ashraf M, Ahmed N, Zhang L (2019) Role of arbuscular mycorrhizal fungi in plant growth regulation: Implications in abiotic stress tolerance. Front. Plant Sci. 10: 1068.

Blackwell P, Joseph S, Munroe P, Anawar HM, Storer P, Gilkes RJ, Solaiman ZM (2015) Influences of biochar and biochar-mineral complex on mycorrhizal colonisation and nutrition of wheat and sorghum. Pedosphere 25: 686-695.

Abdel Latef AA, Chaoxing HJ (2014) Does the inoculation with Glomus mosseae improve salt tolerance in pepper plants? Plant Grow. Regul. 33: 644-653.
Bolandnazar S, Aliasgarzad N, Neishabury MR, Chaparzadeh N (2007) Mycorrhizal colonization improves onion (Allium cepa L.) yield and water use efficiency under water deficit condition. Sci. Hortic. 114: 11-15.

Cavagnaro TR, Bender SF, Asghari HR, Van Der Heijden MGA (2015) The role of arbuscular mycorrhizas in reducing soil nutrient loss. Trends. Plant Sci. 20: 283-290.

Cella JH, Watson J (2000) Manual of laboratory test, 1st Indian Ed. A.I.T.B.S. Publishers and Distributors, New Delhi, India, 265 p.

Chen S, Zhao H, Zou C, Li Y, Chen Y, Wang Z, Jiang Y, Liu A, Zhao P, Wang M, Ahammed GJ (2017) Combined inoculation with multiple arbuscular mycorrhizal fungi improves growth, nutrient uptake and photosynthesis in cucumber seedlings. Front. Microbiol. 8: 2516.

Cyril CN, Kehinde OO, Olanrewaju AD, David SD, Aderemi-Williams O (2014) Vegetative growth and yield response of Amaranthus cruentus to arbuscular mycorrhizal fungi (AMF), poultry manure (PM), combination of AMF-PM and inorganic fertilizer. Am. J. Exp. Agric. 4: 665-673.

Deleke OA, Akomolafe GF (2013) Influence of salinity on soil chemical properties and surrounding vegetation of awa salt mining site, Nasarawa State, Nigeria. Afr. J. Environ. Sci. Technol. 7: 1072-1075.

Ebrahim KE (2014) Role of arbuscular mycorrhizal fungi in fighting soil salinity. PhD Thesis, Royal Holloway-University of London, London, England.

Elmer WH, Pignatelio JJ (2011) Effect of biochar amendments on mycorrhizal associations and Fusarium crown and root rot of asparagus in replant soils. Plant Dis. 95: 960-966.

Evelin H, Giri B, Kapoor R (2011) Contribution of Glomus intra radices inoculation to nutrient acquisition and mitigation of ionic imbalance in NaCl-stressed Trigonella foenum-graecum. Mycorrhiza 22: 1-15.

Evelin H, Kapoor R, Giri B (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Ann. Bot. 104: 1263-1280.

Garg N, Manchanda G (2008) Effect of arbuscular mycorrhizal inoculation of salt-induced nodule senescence in Cajanus cajan (pigeon pea). J. Plant Growth Regul. 27: 115-124.

Gholam C, Foursy A, Fares K (2002) Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. Environ. Exp. Bot. 47: 39-50.

Gomez-Bellot MJ, Ortuño MF, Nortes PA, Vicente-Sánchez J, Bañón S, Sánchez Blanco MJ (2015) Mycorrhizal euonymous plants and reclaimed water: biomass, water status and nutritional responses. Sci. Hort. 186: 61-69.

Hammer EC, Balogh-Brunstad Z, Jakobsen I, Olsson PA, Stipp SLS, Rillig MCA (2014) Mycorrhizal fungus grows on biochar and captures phosphorus from its surfaces. Soil Biol. Biochem. 77: 252-260.

Hammer EC, Forstreuter M, Rillig MC, Kohler J (2015) Biochar increases arbuscular mycorrhizal plant growth enhancement and ameliorates salinity stress. Appl. Soil Ecol. 96: 114-121.

Hashem A, Alqarawi AA, Radhakrishnan R, Al-Arjani AF, Aldehaish HA, Egamberdieva D (2018) Arbuscular mycorrhizal fungi regulate the oxidative system, hormones and ionic equilibrium to trigger salt stress tolerance in Cucumis sativus L. Saudi J. Biol. Sci. 25: 1102-1114.

He L, Li CY, Liu RJ (2017) Indirect interactions between arbuscular mycorrhizal fungi and Spodoptera exigua alter photosynthesis and plant endogenous hormones. Mycorrhiza 27: 525-535.

Heuer B, Yaniv Z, Ravina I (2002) Effect of late salinization of chia (Salvia hispanica), stock (Matthiolatricus pidata) and evening primrose (Oenothera biennis) on their oil content and quality. Ind. Crops Prod. 15: 163-167.

Hirzel J, Donnay D, Fernández C, Meier S, Lagos O, Mejias-Barrera P, Rodriguez F (2018) Evolution of nutrients and soil chemical properties of seven organic fertilizers in two contrasting soils under controlled conditions. Chilean J. Agric. Anim. Sci. ex Agro-Ciencia 34: 77-88.

Hodge A (2000) Microbial ecology of the arbuscular mycorrhiza. Microbiol. Ecol. 32: 91-96.

Hu Y, Schmidhalter U (2002) Limitation of salt stress to plant growth. In Hock B and Elstner CF (Eds.), Plant toxicology, Marcel Dekker Inc., New York, pp 224.

Jaarsma R, Rozemarijn SM, Albertus H (2013) Effect of salt stress on growth, Na+ accumulation and proline metabolism in potato (Solanum tuberosum) cultivars. PLoS ONE 8: 60-183.

Jiang YN, Wang WX, Xie QI, Liu N, Liu LX, Wang DP (2017) Plants transfer lipids to sustain colonization by mutualistic mycorrhizal and parasitic fungi. Sci. 356: 1172-1175.

Ji Xiang L (2010) Assessment of salinity tolerance of Vigna Mungo var. pu-19 using ex vitro and in vitro methods. Asian J. Biotechnol. 2: 73-85.

Kaya C, Higgs D, Kirnak H (2001) Effects of supplementary phosphorus and potassium on physiological development and mineral nutrition of cucumber and pepper cultivars grown at high salinity (NaCl). J. Plant Nutr. 24: 1457-1471.

Kaya C, Kirnak H, Higgs D, Saltati K (2003) Supplementary calcium enhances plant growth and fruit yield in strawberry cultivars grown at high (NaCl) salinity. Hortic. Sci. 26: 807-820.

Kekere O (2014) Role of arbuscular mycorrhizal fungi alleviates chilling stress by boosting redox poise and antioxidant potential of tomato seedlings. J. Plant Growth Regul. 35: 109-120.

Luginbuehl LH, Menard GN, Kurup S, Van Erp H, Radhakrishnan GV, Breakspear A (2017) Fatty acids in arbuscular mycorrhizal fungi are synthesized by the host plant. Science 356: 1175-1178.
Lutts S, Kinet JM, Bouharmont J (1996) NaCl-induced senescence in leaves of rice (Oryza sativa L.) cultivars differing in salinity resistance. Ann. Bot. 78: 389-398.

Mairusia KM, Patel ST (2009) Seed germinability, root and shoot length and vigour index of soybean as influenced by rhizosphere fungi. Karnataka J. Agric. Sci. 22: 1120-1122.

Matrood AAA, Rhouma A (2021) Evaluating eco-friendly botanicals as alternatives to synthetic fungicides against the causal agent of early blight of Solanum melongena. J. Plant Dis. Prot. 128: 1517-1530.

Mickan BS, Abbott LK, Stefanova K, Sollaiman ZM (2016) Interactions between biochar and mycorrhizal fungi in a water-stressed agricultural soil. Mycorrhiza 26: 565-574.

Miller RW, Gardiner DT (2007) Soils in our environment. 9th Ed., Prentice Hall-Incorporated, Upper. Saddle River, New Jersey, pp. 452.

Mufwanzala N, Dikinya O (2010) Impact of poultry manure and its associated salinity on the growth and yield of spinach (Spinacea oleracea) and carrot (Daucus carota). Int. J. Agric. Biol. 12: 489-494.

Mumtaz KM, Ruqaya S, Al-Mas’oudi M, Al-Said F, Khan I (2013) Salinity effects on growth, electrolyte leakage, chlorophyll content and lipid peroxidation in cucumber (Cucumis sativus L.). Intern. Conference Food Agric. Sci. 55: 30-32.

Ortas I (2012) The effect of mycorrhizal fungal inoculation on plant yield, nutrient uptake and inoculation effectiveness under long-term field conditions. Field Crops Res. 125: 35-48.

Pandian K, Subramaniyan P, Gnasekaran P, Chitravathirapillai S (2016) Effect of biochar amendment on soil physical, chemical and biological properties and groundnut yield in rainfed Alfisols of semi-arid tropics. Arch. Agron. Soil Sci. 62: 1293-1310.

Paymaneh Z, Gryndler M, Konvalinková T, Benada O, Borovicka J, Bukovská P, Püschel D, Rezácová V, Sarcheshmehpour M, Jansa J (2018) Soil matrix determines the outcome of interaction between mycorrhizal symbiosis and biochar for Andropogon gerardii growth and nutrition. Front. Microbiol. 9: 2862.

Plassard C, Dell B (2010) Phosphorus nutrition of mycorrhizal trees. Tree Physiol. 30: 1129-1139.

Qiu L, Bi Y, Jiang B, Wang Z, Zhang Y, Zhakypbek Y (2019) Arbuscular mycorrhizal fungi ameliorate the chemical properties and enzyme activities of rhizosphere soil in claimed mining subsidence in northwestern China. J. Arid Land 11: 135-147.

Revell KT, Maguire RO, Agblevor FA (2012) Influence of poultry litter biochar on soil properties and plant growth. Soil Sci. 177: 402-408.

Rhouma A, Hamouda N, Bessadok S (2017) Assessment of the pH of water and sediments at Ramsar Sites in Gabes city, Tunisia. J. Ecobiotecnol. 9: 13-17.

Rhouma A, Salem IB, M’hamdi M, Boughalleb-M’Hamdi N (2019) Relationship study among soils physico-chemical properties and Monosporascus cannonballus ascospores densities for cucurbit fields in Tunisia. Eur. J. Plant Pathol. 153: 65-78.

Rouphael Y, Franken P, Schneider C, Schwarz D, Giovannetti M, Agnolucci M (2015) Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. Sci. Hortic. 196: 91-108.

Sallam BN, Lu T, Yu H, Li Q, Sarfraz Z, Iqbal MS, Khan S, Wang H, Liu P, Jiang W (2021) Productivity enhancement of cucumber (Cucumis sativus L.) through optimized use of poultry manure and mineral fertilizers under greenhouse cultivation. Horticulturae 7: 256.

Saxena B, Giri B, Shukla K (2017) Arbuscular mycorrhizal fungi and tolerance of salt stress in plants. In Wu QS (Eds.), Arbuscular mycorrhizas and stress tolerance of plants. Springer Nature Singapore Pte Ltd., Singapore, pp. 73.

Selosse MA, Strullu-Derrien C, Martin FM, Kamoun S, Kenrick P (2015) Plants, fungi and oomycetes: a 400-million years affair that shapes the biosphere. New Phytol. 206: 501-506.

Selvaraj T, Chellappan P (2006) Arbuscular mycorrhizae: a diverse personality. J. Cent. Eur. Agric. 2: 349-358.

Shan SW (2009) Enhanced phytoremediation of salt-impacted soils using plant growth-promoting rhizobacteria (PGPR). M.Sc. Thesis, University of Waterloo, Canada.

Sheng M, Tang M, Chan H, Yang B, Zhang F, Huang Y (2008) Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. Mycorrhiza 18: 287-296.

Shi Q, Bao Z, Zhu Z, Ying Q, Qian Q (2006) Effects of different treatments of salicylic acid on heat tolerance, chlorophyll fluorescence, and antioxidant enzyme activity in seedlings of Cucumis sativa L. Plant Growth Regul. 48: 127-135.

Shou-Jun Y, Zhong-Lan Z, Yuan-Xia X, Zhi-Fen Z, Shu-Yi S (2014) Arbuscular mycorrhizal fungi increase salt tolerance of apple seedlings. Bot. Stud. 55: 4-7.

Sidker S, Joardar J (2019) Biochar production from poultry litter as management approach and effects on plant growth. Int. J. Recycl. Org. Waste Agric. 6: 48-57.

Sistani KR, Simmons JR, Jn-Baptiste M, Novak JM (2019) Poultry litter, biochar, and fertilizer effect on corn yield, nutrient uptake, N2O and CO2 emissions. Environ. 6: 55.

Sofy M, Mohamed H, Dawood M, Abu-Elsaoud A, Soliman M (2021a) Integrated usage of arbuscular mycorrhizal and biochar to ameliorate salt stress on spinach plants. Arch. Agron. Soil Sci.

Sofy MR, Aboseidah AA, Heneidak SA, Hoda RA (2021b) ACC deaminase containing endophytic bacteria ameliorate salt stress in Pisum sativum through reduced oxidative damage and induction of antioxidative defense systems. Environ. Sci. Pollut. Res. 28: 40971-40991.

Sollaiman ZM, Abbott LK, Murphy DV (2019) Biochar phosphorus concentration dictates mycorrhizal colonisation, plant growth and soil phosphorus cycling. Sci. Rep. 9: 5062.

Sollaiman ZM, Shafii MI, Beamont E, Anawar HM (2020) Poultry litter biochar increases mycorrhizal colonisation, soil fertility and cucumber yield in a fertigation system on sandy soil. Agriculture 10: 480.
Sun Z, Song J, Xin X, Xie X, Zhao B (2018) Arbuscular mycorrhizal fungal proteins are involved in arbuscule formation and responses to abiotic stresses during AM symbiosis. Front. Microbiol. 5: 9-19.

Talaat NB, Shawky BT (2014) Protective effects of arbuscular mycorrhizal fungi on wheat (Triticum aestivum L.) plants exposed to salinity. Environ. Exp. Bot. 98, 20-31.

Tanji KK (2002) Salinity in the soil environment. In Läuchli A and Lüttge U (Eds.), Salinity: environment-plants-molecules. Netherlands: Kluwer Academic Publishers, pp. 21-51.

Teixeira M, Carvalho IS (2009) Effects of salt stress on purslane (Portulaca oleracea) nutrition. Ann. Appl. Biol. 154: 77-86.

Uddin MK, Shamsuzzaman S, Zi LQ, Mohdselamat MH (2017) Effects of salinity on growth, antioxidant contents and proximate compositions of sabah snake grass (Clinacanthus Nutans (Burm. F.) Lindau.). Bangladesh J. Bot. 46: 263-269.

Uzun S, Uzun O, Kaplan M, Ibas AI (2013) Response of bitter vetch lines to salt stress. Bulg. J. Agric. Sci. 19: 1061-1067.

Wang Y, Wang M, Li Y, Wu A, Huang J (2018) Effects of arbuscular mycorrhizal fungi on growth and nitrogen uptake of Chrysanthemum morifolium under salt stress. PLoS One 13: e0196408.

Wu DC, Ré DB, Nagai M, Ischiropoulos H, Przedborski S (2006) The inflammatory NADPH oxidase enzyme modulates motor neuron degeneration in amyotrophic lateral sclerosis mice. Proc. Natl. Acad. Sci. USA 103: 12132-12137.

Wu QS, Zou YN, Liu W (2010a) Alleviation of salt stress in citrus seedlings inoculated with mycorrhiza: changes in leaf antioxidant defense systems. Plant Soil Environ. 56: 470-475.

Wu QS, Zou YN, He XH (2010b) Contributions of arbuscular mycorrhizal fungi to growth, photosynthesis, root morphology and ionic balance of citrus seedlings under salt stress. Acta. Physiol. Plant 32: 297-304.

Yadav K, Aggarwal A, Singh N (2013) Arbuscular mycorrhizal fungi (AMF) induced acclimatization, growth enhancement and colchicine content of micropropagated Gloriosa superba L. plantlets. Ind. Crop. Prod. 45: 88-93.

Yang Y, Tang M, Suligne R, Chen H, Tian S, Ban Y (2014) Arbuscular mycorrhizal fungi alter fractal dimension characteristics of Robinia pseudoacacia L. seedlings through regulating plant growth, leaf water status, photosynthesis, and nutrient concentration under drought stress. J. Plant Growth Regul. 33: 612-625.

Zhang Z, Dong X, Wang S, Pu X (2020) Benefits of organic manure combined with biochar amendments to cotton root growth and yield under continuous cropping systems in Xinjiang, China. Sci. Rep. 10: 4718.

Zhu JK (2007) Plant salt stress. John Wiley and Sons Ltd., pp. 22-24.

Zuccarini P, Okurowska P (2008) Effects of mycorrhizal colonization and fertilization on growth and photosynthesis of sweet basil under salt stress. J. Plant Nutr. 31: 497-513.