Effect of Vesicular Arbuscular Mycorrhizal (VAM) Fungus and Rock-Phosphate Application on the Growth and Biomass of *Moringa oleifera* Lam. Seedlings under Salinity Stress

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**ABSTRACT**

Salinity is a devastating environmental stress factor that severely affects plant growth and development. Soil salinity often hinders plant productivity in both natural and agricultural settings. Vesicular Arbuscular mycorrhizal fungal (VAM) symbionts can mediate plant stress responses by enhancing salinity tolerance. Experiments were conducted in a greenhouse at the nursery of the Experimental Station of Forestry and Wood Technology Dept., Faculty of Agriculture, University of Alexandria, Abies region, Alexandria, from June, 2017 to May, 2018 and repeated at the same time in the second season. The obtained results showed that the inoculation with VAM and addition of RP led to enhance the growth significantly, in terms of survival, shoot height, shoot root ratio, root dry weight, shoot dry weight and total dry weight and minerals of the leaves of *M. oleifera* (N, P and K%) compared with the uninoculated ones. Chlorophyll a of *M. oleifera* was affected by salinity. Na Cl treatments caused a decrease in chlorophyll a and chlorophyll b content in both seasons. The largest increases in plants nutrient uptake (N, P and K) and decreasing in Na were observed with the VAM+RP treatment. The inoculated seedlings with VAM induced the highest value in Proline content in the first and second seasons compared with the uninoculated ones. The present study concluded that (*M. oleifera* Lam.) could tolerate salt concentration up to 171.1 mM in the presence of mycorrhiza. It is recommended; however, to inoculate the seedlings with VAM and (1g/kg soil) rock-phosphate application to enhance its growth and mitigate salinity stress.

Key words: Salinity, Moringa, Rock-phosphate, Proline, Mycorrhiza and VAM.

**INTRODUCTION**

Salinity is a devastating environmental stress factor that severely affects plant growth and development (Barnawal et al., 2014). Soil salinity is rapidly increasing with an estimated addition of 0.3–1.5 million ha of farmland annually, thereby decreasing crop production by more than 20% (Porcel et al., 2012; and Food and Agriculture Organization [FAO], 2015). It also renders another 20–46 million ha with decreased capacity for production. Nevertheless, the earth is home to 7.7 billion people with addition of 83 million people every year at the rate of 1.09% (United Nations [UN], 2018). At the global level, particularly in arid and semiarid regions, salt alters a wide range of metabolic processes, culminating in stunted growth, and minimized enzyme activities and biochemical constituents (Muthukumarasamy and Panneerselvam, 1997). Salinity, furthermore is considered an important constraint, and approximately 7% of global land has suffered from high salinity, making it unarable (Sheng et al., 2008 and Ruiz-Lozano et al., 2012). Physiologically, salinity reduces enzymes activities and plant growth through osmotic as well as ionic constraints of major physiological and biochemical reactions (Ahmad, 2010; Porcel et al., 2012; Abd_Allah et al., 2015).

Proline accumulation has been first observed in wilting perennial plants (Kemble and MacPherson, 1954) and was later found to be one of the common physiological responses of higher plants when they are exposed to a number of environmental stresses (Verbruggen and Hermans 2008). Proline accumulation has been reported in plants exposed to high salinity (Armengaud, et al., 2004). Proline is the most common osmolyte in plants under stress conditions (Hasegawa et al., 2000) and act as a mediator of osmotic adjustment (Ashraf and Foolad, 2007) and serves as a hydroxyl radical scavenger (Alia et al., 1995). There are accumulating evidences that the production of reactive oxygen species (ROS) is a major damaging factor in plants exposed to different environmental stresses, including salinity (Hernandez et al., 1995). Peroxidase (POX) and catalase (CAT) are involved in the defense mechanisms of plants in response to pathogens by their participation in cell wall reinforcement. Cells under salt stress initially accumulate salts as free osmotica, however, a toxic specific ion effect appears once a certain threshold level of Na and/ or Cl accumulation has been reached. An excess of these ions may alter membrane integrity, enzymatic activity, protein and

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nucleic metabolism (Hasegawa et al., 2000; Zhu 2001, Zhu and Liming 2002 and Mansour and Salama, 2004).

Plants under stress produce some defense mechanisms to protect themselves from the harmful effect of oxidative stress. ROS scavenging is one among the common defense response against abiotic stresses. ROS scavenging depends on the detoxification mechanism provided by an integrated system of non-enzymatically reduced molecules like ascorbate, glutathione and enzymatic antioxidants (Prochazkova et al., 2001; Shrivali et al., 2003). The primary antioxidant enzyme which converts superoxide to H$_2$O and oxygen is superoxide dismutase (SOD) (Alscher et al., 2002). The main enzyme involved in H$_2$O scavenging is also catalase, which decomposes H$_2$O$_2$ to water and oxygen. SOD and CAT are considered as key components in the antioxidant response system as they regulate the cellular concentration of O$_2^-$ and H$_2$O$_2$ (Van Breusegem et al., 2001).

*Moringa oleifera* in Pakistan named as sohanjna is a miracle tree having tremendous uses like phytostatcides, afforestation, medicines, water purification, biogas, vegetable etc (Wasif, et al., 2012). It is naturally found in diverse habitats with an altitude ranging from 600-1800 m (Jama and Yucel 1989). Recently, many uses of moringa have been highlighted and farmers are taking interest to cultivate it as field crop for fodder and vegetable production and as forestry plantation (Chen and Bin, 2020).

Vesicular arbuscular mycorrhizal (VAM) fungi are considered as beneficial symbiotic associations with most plants and play a main role in plant growth under various conditions by modifying the root system and enhancing mobilization and the uptake of several essential elements. They have also been reported to stimulate plant stress tolerance by enhancing systems of enzymatic and nonenzymatic antioxidant defense (Wu et al., 2014; Ahmad et al., 2015. There is a body of evidence for the role of mycorrhizal fungi in disease resistance of the plant per se (Zeng, 2013). It is known that VAM fungi can increase plant growth and productivity under different conditions, including various soil stresses (Hildebrandt et al., 2007; Miransari et al., 2008; Evelin et al., 2009 and 2011 and Dudhane et al., 2011).

Herewise, this study aimed at pinpointing the effect of mycorrhizal fungi and rock phosphate fertilization on the growth of *Moringa oleifera* under salinity stress and determination of mineral content (%) in the treated leaves of *Moringa oleifera* seedlings.

### MATERIALS AND METHODS

#### 1. Plant material and growth conditions

Experiments were conducted in a greenhouse at the nursery of the Experimental Station of Forestry and Wood Technology Dept., Faculty of Agriculture, University of Alexandria, Abies region, Alexandria, from June, 2017 to May, 2018 and repeated at the same time in the second season. Seeds were sown on 18th, July 2017 and 2018. Seeds of *Moringa oleifera* were germinated in a soil mixture of perlite, sand, peatmoss and vermiculite (1:1:1:1 v/v). Phosphorous as rock phosphate was added at the rate of 0.0, 1.0 and 2.0g/ kg soil. *Moringa* seedlings were 40 - days - old. Half of the total pots were inoculated with the mycorrhizal fungus, *Glomus fasciculatum* as *Moringa* seedlings were two months old. The VAM inoculum consisted of soil, clamydospores (Ca 50 spores g$^{-1}$ inoculum). To Furnish the same soil conditions, control plants were inoculated, yet with sterilized inocula. One month after the artificial inoculation with mycorrhizal fungus, salinization treatments were conducted using five salinity levels (0, 42.78, 85.56, 128.24 and 171.1 (mili mole) mM Na Cl).

#### 2. Experimental design

The experimental design consisted of thirty treatments having non- AM inoculated and AM inoculated with three phosphorus levels (0, 0.1g and 2 g/ kg soil) and five salinity levels (NaCl: 0, 42.78, 85.56, 128.24 and 171.1 (mili mole) mM. Pots were arranged in a completely randomized design. The split-split plot technique was used in analyzing the data obtained, where the main plot was for phosphorus fertilization, the sub plot was for salinity levels and the sub-sub plot was for inoculation with symbiotic agent.

#### Table 1. Outline of the source of variation and its degree of freedom (d.f) of the experiment used.

| Source of variance | d.f |
|--------------------|-----|
| Replicates         | 3   |
| A                  | 2   |
| Error a            | 6   |
| B                  | 4   |
| AB                 | 8   |
| Error b            | 36  |
| C                  | 1   |
| AC                 | 2   |
| BC                 | 4   |
| ABC                | 8   |
| Error c            | 45  |
| Total              | 119 |

The data obtained were statistically analyzed according to Snedecor (1956) using SAS ver. 9.1.3 (2007). Four replications were used for each treatment i.e. total 120 pots. Three months after germination, homogenized
seedlings were selected for the experimental study. Treated seedlings were monitored, cared and all observations were recorded. In addition, root samples were examined for presence of VAM, if any. Growth parameters, notably, shoot height and abnormal symptoms were recorded after one month, seedlings were harvested for further analysis.

3. Ultrastuctural examination of infected feeder roots with VAM

Feeder roots samples were collected, washed free from debris, cut into small pieces (3 mm length), then soaked in a chain of ten concentrations of ethanol solution, 10, 20, 30, ----, 100%, then in xylol. The specimens were soaked in each concentration for 1.0 hour, then dried and fixed for scanning electron microscope (SEM) examination, according to the method described by Hayat, (1991).

4. Morphological parameters

The analyzed morphological parameters, survival (%), shoot height (SH), shoot dry weight (SDW), root dry weight (RDW), total dry weight (TDW) and shoot root ratio (SRR) were recorded.

5. Biochemical parameters.

Proline colorimetrically determined according to Marín et al., 2009. The protocol for Chlorophyll a and b was applied to determine its content according to Nagata and Yamashita, 1992, while mineral contents of plants were determined in all seedlings according to Chapman and Pratt, 1961 and Olsen and Sommers, 1982.

Table 2. The chemical analysis of the experimental soil.

| Characteristics          | Value |
|--------------------------|-------|
| pH (1 soil : 2.5 d.w.)   | 8.6   |
| E.C. (mmohs/cm)          | 11.5  |
| Anion (mq/100 g soil)    |       |
| Cl⁻                     | 103   |
| HCO₃⁻                   | 2.4   |
| SO₄²⁻                   | 26.4  |
| CO₃⁻                    | ----- |
| Cations (mq/100 g soil)  |       |
| Mg ++                   | 22.3  |
| Na +                    | 91.2  |
| Ca ++                   | 18.3  |
| K +                     | 1.9   |

RESULTS

1. Mycorrhization

The scanning electron microscope examination has revealed the colonization of extrametrical hyphae of VAM of rootlet cortex cells of inoculated seedlings with VAM as shown in (Fig.1). it has also indicated that the feeder roots of Moringa oleifera contained arbuscules of Glomus fasiculatum and its internal hyphae (Fig. 2).

2. Healthy and growth parameters

Growth parameters including survival (%), shoot height (cm), shoot dry weight (g), root dry weights (g), total dry weight (g) and shoot root ratio of Moringa oleifera Lam. seedlings in both seasons are shown in Tables (4, 5, 6, 7 and 8). The present study showed that (Moringa oleifera Lam.) could tolerate salt concentrations up to 171.1 mM in presence of Mycorrhiza. Negative relationship was obtained between salt stress degree and plant growth parameters during the growing seasons.

Fig. 1. Scanning electron micrograph indicates root surface (RS) penetrated by extrametrical hyphae of VAM fungus (EH)
Fig. 2. (A) Scanning electron micrograph (SEM) indicates feeder root of *Moringa oleifera* contained Arbuscules of *Glomus fasciculatum*. IH: Internal hyphae, Ar: Arbuscule and CW: Cell wall. (B) Mature parenchymatous cells with starch granules (SG) in the cortex of feeder root cell.

2.1. Survival (S) (%)

Regardless of the impact of salinity and inoculation with VAM, there is no significant differences among RP level applied in terms of S. There were significant differences among the impacts of salinity levels. However, the lowest S was obtained in the seedlings treated with S5 in both seasons, (62.50 and 70.83 % for first and second season, respectively) (Table 3).

The inoculation with VAM has also brought about the highest S in both seasons (96.67 and 98.33 % for first and second season, respectively) (Table 3).

| Rock phosphate RP | Salinity level (ppm) | VAM inoculation | RP*S | RP | S | VAM inoculation | RP*S | RP | S |
|------------------|---------------------|----------------|------|----|---|----------------|------|----|---|
|                  |                     |                |      |    |   |                |      |    |   |
| Rp1              | 0.0g                | S1             | 100  | 100| 100| S1             | 100  | 100| 100|
|                  |                     | S2             | 100  | 100| 100| S2             | 100  | 100| 100|
|                  |                     | S3             | 100  | 100| 100| S3             | 100  | 100| 100|
|                  |                     | S4             | 75   | 100| 87.5| S4             | 100  | 100| 100|
|                  |                     | S5             | 75   | 100| 87.5| S5             | 75   | 100| 87.5|
| Rp1*VAM          | 90.00               | 90.00          | 95.0 |    |    | 95.00          | 95.00| 97.50|
|                  |                     | S1             | 100  | 100| 100| S1             | 100  | 100| 100|
|                  |                     | S2             | 100  | 100| 100| S2             | 100  | 100| 100|
|                  |                     | S3             | 100  | 100| 100| S3             | 100  | 100| 100|
|                  |                     | S4             | 50   | 100| 75 | S4             | 75   | 100| 87.5|
|                  |                     | S5             | 50   | 75 | 62.5| S5             | 75   | 100| 87.5|
| Rp2*VAM          | 90.00               | 90.00          | 92.50|    |    | 95.00          | 95.00| 97.50|
|                  |                     | S1             | 100  | 100| 100| S1             | 100  | 100| 100|
|                  |                     | S2             | 100  | 100| 100| S2             | 100  | 100| 100|
|                  |                     | S3             | 100  | 100| 100| S3             | 100  | 100| 100|
|                  |                     | S4             | 75   | 100| 87.5| S4             | 75   | 100| 87.5|
|                  |                     | S5             | 0.00 | 75 | 37.5| S5             | 0.00 | 75 | 37.5|
| Rp3*VAM          | 75.00               | 75.00          | 85.0 |    |    | 85.00          | 75   | 95.00| 85.00|
|                  |                     | S1             | 100  | 100| 100| S1             | 100  | 100| 100|
|                  |                     | S2             | 100  | 100| 100| S2             | 100  | 100| 100|
|                  |                     | S3             | 100  | 100| 100| S3             | 100  | 100| 100|
|                  |                     | S4             | 83.33 | 100| 91.67| S4             | 91.67| 100| 95.83|
|                  |                     | S5             | 41.67 | 83.33| 62.50| S5             | 62.50| 100| 70.83|
| S*VAM            | 85.00               | 85.00          | 88.33|    |    | 88.33          | 85.00| 98.33|
|                  |                     | S1             | 100  | 100| 100| S1             | 100  | 100| 100|
|                  |                     | S2             | 100  | 100| 100| S2             | 100  | 100| 100|
|                  |                     | S3             | 100  | 100| 100| S3             | 100  | 100| 100|
|                  |                     | S4             | 83.33 | 100| 91.67| S4             | 91.67| 100| 95.83|
|                  |                     | S5             | 41.67 | 83.33| 62.50| S5             | 62.50| 100| 70.83|
| VAM              | 85.00               | 85.00          | 88.33|    |    | 88.33          | 85.00| 98.33|
| LSD at           | RP= ------         | S= 6.52        | VAM = 9.62 |    |    | VAM = 9.62     | RP= ------         | S= 5.075 | VAM = 9.88 |
|                  | 0.05               | RP*S* = 2.0523 | RP*S*VAM= 13.54 |    |    | RP*S* = 14.16 |
2.2. Shoot height (SH) (cm)

Comparing the impact of Rock Phosphate (RP) levels, non significant differences were observed among RP level applied in terms of SH. There were significant differences among the impact of salinity levels, the highest SH was obtained in the seedlings treated with S₁ in both seasons (36.1 and 47.9cm for first and second season, respectively), whilst the lowest value was found in those treated with S₅ in both seasons (26.3 and 31.3cm for first and second season, respectively) (Table 4).

As for the effect of inoculation with the symbiotic agent, it was found that the inoculated seedlings with VAM have exhibited the highest SH in both seasons (36.1 and 37.2 cm for first and second season, respectively) (Table 4).

Furthermore, the statistical analysis has also revealed the significant interaction between the impact of Rock-phosphate (RP) application and VAM inoculation and the triple interaction among RP application, salinity levels and VAM inoculation. The inoculated seedlings with VAM, fertilized with RP₂ and applied with S₂ displayed the highest value of SH in the first season, since it was (49.9cm), yet in the second season the inoculated seedlings with VAM, unfertilized with RP₂ and applied with S₂ displayed the highest value of SH, since it was (49.8cm) (Table 4).

2.3. Shoot dry weight (SDW) (g)

It was found that the seedlings which fertilized with RP₃ displayed the highest SDW in both growing seasons (3.1162 and 3.2813g for first and second season, respectively) (Table 5).

Table 4. Shoot height (cm) of inoculated and uninoculated seedlings of *Moringa oleifera* Lam. with VAM, unfertilized and fertilized with RP under five levels of salinity

| Rock phosphate | Salinity level (ppm) | VAM inoculation | RP*S | RP | S | VAM inoculation | RP*S | RP | S |
|----------------|---------------------|----------------|------|----|---|----------------|------|----|---|
|                |                     | C | VAM |      | C | VAM |      |      |    |    |
| Rp₁ 0.0g       | S₁                  | 29.4| 37.7| 33.6| 38.5| 47.1| 42.8|      |    |    |
|                | S₂                  | 25.3| 40.4| 32.9| 34.5| 49.8| 42.2|      |    |    |
|                | S₃                  | 23.6| 38.6| 31.1| 32.9| 47.9| 40.4|      |    |    |
|                | S₄                  | 23.6| 35.8| 29.7| 32.9| 45.2| 39.1|      |    |    |
|                | S₅                  | 13.0| 30.3| 21.7| 22.3| 39.8| 31.1|      |    |    |
| Rp₁*VAM        | S₁                  | 23.0| 36.6| 29.8| 32.2| 46.0| 39.1|      |    |    |
|                | S₂                  | 31.0| 37.6| 34.3| 37.9| 37.2| 37.6|      |    |    |
| Rp₂ 1.0g       | S₁                  | 29.4| 49.9| 39.7| 36.4| 48.7| 42.6|      |    |    |
|                | S₂                  | 24.4| 41.7| 33.1| 31.8| 40.5| 36.2|      |    |    |
|                | S₃                  | 22.8| 33.5| 28.2| 30.3| 32.3| 31.3|      |    |    |
|                | S₄                  | 0.0 | 27.7| 13.9| 0.0 | 26.6| 13.8|      |    |    |
| Rp₂*VAM        | S₁                  | 21.5| 38.1| 29.8| 27.3| 37.1| 32.3|      |    |    |
|                | S₂                  | 29.4| 36.8| 33.1| 20.6| 31.6| 26.1|      |    |    |
| Rp₃ 2.0g       | S₁                  | 29.4| 41.3| 35.4| 21.6| 36.1| 28.9|      |    |    |
|                | S₂                  | 26.7| 27.6| 27.2| 19.3| 22.6| 21.0|      |    |    |
|                | S₃                  | 31.1| 34.0| 32.6| 23.1| 28.9| 26.0|      |    |    |
|                | S₄                  | 0.0 | 28.5| 14.3| 0.0 | 23.5| 11.8|      |    |    |
| Rp₃*VAM        | S₁                  | 23.3| 33.6| 28.5| 16.9| 28.5| 22.7|      |    |    |
|                | S₂                  | 29.9| 37.4|      | 33.7| 32.3| 38.6| 35.5|    |    |
|                | S₃                  | 28.3| 43.9|      | 36.1| 30.8| 44.9| 47.9|    |    |
| S*VAM          | S₁                  | 24.9| 36.0|      | 30.4| 28.0| 37.0| 32.5|    |    |
|                | S₂                  | 25.8| 34.4|      | 30.1| 28.8| 35.5| 32.1|    |    |
|                | S₃                  | 22.7| 29.9|      | 26.3| 25.5| 37.2| 31.3|    |    |
| VAM            |                    | 22.6| 36.1|      | 25.5| 37.2|      |    |    |
| LSD at RP:------S= 2.10 | VAM = 2.46 | RP:------S= 2.33 | RP*VAM =4.37 | RP*S*VAM = 5.23 | VAM = 2.46 | RP*VAM = 4.41 | RP*S*VAM = 5.63
Table 5. Shoot dry weight (g) of inoculated and uninoculated seedlings of *Moringa oleifera* Lam. with VAM, unfertilized and fertilized with RP under five levels of salinity

| Rock phosphate RP | Salinity level (ppm) | First season | Second season | First season | Second season |
|-------------------|----------------------|--------------|---------------|--------------|---------------|
|                   | VAM inoculation      | C | VAM | RP*S | RP | S | VAM inoculation | RP*S | RP | S |
| Rp1 0.0g          | S1 1.831            | 2.871 | 2.351 | 1.607 | 3.528 | 2.5675 |
|                   | S2 2.026            | 3.683 | 2.8545 | 1.779 | 4.528 | 3.1535 |
|                   | S3 0.86             | 2.827 | 1.8435 | 0.755 | 3.475 | 2.115 |
|                   | S4 0.791            | 2.643 | 1.717 | 0.695 | 3.249 | 1.972 |
|                   | S5 0.413            | 1.679 | 1.046 | 0.363 | 2.064 | 1.2135 |
| Rp1*S*VAM         | S1 1.1842           | 2.7406 | 1.9624 | 1.0398 | 3.3688 | 2.2043 |
|                   | S2 3.391            | 4.777 | 4.084 | 3.262 | 4.424 | 3.752 |
|                   | S3 3.672            | 2.416 | 3.044 | 3.532 | 2.145 | 2.8385 |
|                   | S4 1.585            | 2.015 | 1.8 | 1.525 | 1.789 | 1.657 |
|                   | S5 1.202            | 0.749 | 0.9755 | 1.156 | 0.665 | 0.9105 |
| Rp1*S*VAM         | S1 1.97             | 2.3228 | 2.1464 | 1.895 | 2.0626 | 1.9788 |
|                   | S2 3.272            | 2.687 | 2.9795 | 1.953 | 3.302 | 2.6275 |
|                   | S3 3.095            | 6.695 | 4.895 | 2.789 | 8.229 | 5.509 |
|                   | S4 1.875            | 6.327 | 4.101 | 2.639 | 6.19 | 4.4145 |
|                   | S5 2.239            | 3.705 | 2.972 | 1.599 | 4.354 | 3.0765 |
| Rp1*S*VAM         | S1 2.0962           | 4.1362 | 3.1162 | 1.796 | 4.7666 | 3.2813 |
|                   | S2 3.072            | 2.067 | 1.8829 | 1.3644 | 2.2144 | 1.7894 |
|                   | S3 1.7196           | 2.3964 | 2.058 | 1.5856 | 2.7804 | 2.183 |
| S*VAM             | S1 0.864            | 2.2338 | 1.5489 | 0.9838 | 2.2908 | 1.6373 |
|                   | S2 0.8464           | 1.4194 | 1.1329 | 0.69 | 1.6936 | 1.1918 |
|                   | S3 0.0826           | 0.9206 | 0.5016 | 0.0726 | 1.0188 | 0.5457 |
| VAM               | 1.750              | 3.066 | 1.577 | 3.399 |

LSD at 0.05 RP: 0.02365 S = 0.5462 VAM = 1.2632 RP= 0.02451 S = 0.73254 VAM*S =0.56841 RP*S*VAM= 3.233

2.4. Root dry weight (RDW) (g)

There were significant effects of the salinity level. However, seedlings amended with S1 displayed the highest RDW in both seasons (6.202 and 6.44 for first and second season, respectively). (Table 6).

As for the impact of inoculation of mycorrhizal fungus, there were significant differences among uninoculated seedlings (control) and inoculated ones with VAM, since the inoculated seedlings displayed the highest value of RDW in the both seasons, respectively, (7.867 and 8.168g for first and second season, respectively) (Table 6).

Upon the triple interaction, there was a significant interaction among the three studied factors. However, the highest TDW was obtained in the inoculated seedlings which applied with Rp1 and amended with S2 in both seasons (19.66 and 20.42g for first and second season, respectively). (Table 6).
Table 6. Root dry weight (g) of inoculated and uninoculated seedlings of *Moringa oleifera* Lam. with VAM, unfertilized and fertilized with RP under five levels of salinity

| Rock phosphate | Salinity level (ppm) | First season | Second season |
|----------------|----------------------|--------------|---------------|
|                | VAM inoculation | | | |
|                | C | VAM | RP*S | RP | S | | | | |
|                | C | VAM | | | | | | | |
| Rp1 | | | | | | | | | |
| 0.0g | S1 | 9.91 | 6.38 | 8.145 | 10.29 | 6.62 | 8.455 |
| | S2 | 4.86 | 15.19 | 10.025 | 5.05 | 15.77 | 10.41 |
| | S3 | 13.85 | 3.42 | 8.635 | 14.38 | 3.55 | 8.965 |
| | S4 | 3.41 | 12.64 | 8.025 | 3.54 | 13.13 | 8.335 |
| | S5 | 12.45 | 3.75 | 8.1 | 12.93 | 3.9 | 8.415 |
| Rp2 | | | | | | | | | |
| 1.0g | S1 | 8.896 | 8.276 | 8.586 | 9.238 | 8.594 | 8.916 |
| | S2 | 9.03 | 4.34 | 6.685 | 9.37 | 4.51 | 6.94 |
| | S3 | 4.45 | 8.66 | 6.555 | 4.62 | 8.99 | 6.805 |
| | S4 | 12.3 | 7.14 | 9.72 | 12.77 | 7.42 | 10.995 |
| | S5 | 3.31 | 2.4 | 2.855 | 3.44 | 2.49 | 2.965 |
| Rp3 | | | | | | | | | |
| 2.0g | S1 | 5.818 | 4.968 | 5.393 | 6.04 | 5.158 | 5.599 |
| | S2 | 11.27 | 8.77 | 10.02 | 11.7 | 9.1 | 10.4 |
| | S3 | 6.51 | 19.66 | 13.085 | 6.76 | 20.42 | 13.59 |
| | S4 | 12.51 | 12.8 | 12.655 | 12.99 | 13.29 | 13.14 |
| | S5 | 2.27 | 5.36 | 3.815 | 2.36 | 5.56 | 3.96 |
| Rp3| | | | | | | | | |
| S*VAM | | | | | | | | | |
| 3.0g | S1 | 6.042 | 3.898 | 4.97 | 6.272 | 4.046 | 5.159 |
| | S2 | 3.164 | 8.702 | 5.933 | 3.286 | 9.036 | 6.161 |
| | S3 | 7.732 | 4.672 | 6.202 | 8.028 | 4.852 | 6.44 |
| | S4 | 1.798 | 4.08 | 2.939 | 1.868 | 4.236 | 3.052 |
| | S5 | 2.49 | 2.25 | 2.37 | 2.586 | 2.336 | 2.461 |
| VAM | | | | | | | | | |
| | 7.0753 | 3 | 7.3467 | 8.1687 | | |
| LSD at 0.05 | RP= ------- | S= 0.02563 | VAM = 0.000315 | RP= ------- | S= 0.00415 | VAM = 0.00036 | RP*S*VAM= 3.452 | |

2.5. Total dry weight (TDW) (g)

Seedlings treated with S2 displayed the highest TDW in both seasons (7.991 and 8.344 g for first and second season, respectively), while the seedlings which applied with S5 recorded in both seasons (2.872 and 3.007 g for first and second season, respectively), (Table 7).

As for the effect of inoculation with symbiotic agent, there were significant differences among uninoculated seedlings (control) and inoculated ones with symbiotic agent under study. It was found that the inoculated seedlings had the highest TDW in both seasons (10.934 and 11.568 g), respectively (Table 7).

Finally, there was a significant interaction among the three factors studied. It can be observed that the highest TDW was obtained in the inoculated seedlings, applied with Rp3 and amended with S3 in both seasons (26.335 and 28.649 g for first and second season, respectively), (Table 7).

2.6. Shoot/ root ratio (SRR)

Application of RP the seedlings which treated with RP3 induced that the highest SRR in both seasons, (0.522 and 0.375 for first and second season, respectively), (Table 8).

Upon the significant interaction between RP application and VAM inoculation it was found that the seedlings inoculated with VAM and treated with level RP2 displayed the highest SRR (0.468) in the first season, yet in the second season the inoculated seedlings with mycorrhiza and treated with RP3 displayed the highest SRR, since it was 0.443. (Table 8).
Considering the significant triple interaction among the studied factors, the inoculated seedlings which were amended with RP2 and untreated with salt have displayed the highest SRR (1.101, 0.941 for first and second season, respectively) (Table 8).

4. Chemical analysis:

4.1. Chlorophyll a (Chl a) and Chlorophyll b (Chl b) (mg/100g).

Chlorophyll a of *Moringa oleifera* was affected by salinity (Table 9). NaCl treatments caused a decrease in chlorophyll a and chlorophyll b content in both seasons, since it was 67.49mg/100g at 128.24 mM and 54.81mg/100 at 171.1 mM in the first season for chlorophyll a and as 72.25mg/100g at 128.24 mM and 58.79mg/100 at 171.1 mM in the second season. Similar responses in chlorophyll b were observed (Table 10).

According to the significant interaction between salinity and RP treatments, the addition of RP$_1$ + S$_1$ gave the highest chlorophyll a and (75.88 and 81.24mg/100g in the first and second season, respectively) and chlorophyll b (112.95 and 126.69mg/100g in the first and second season, respectively) (Tables 9 and 10). Under salinity stress, photosynthetic pigments were reduced due to accumulation of higher concentrations of Na+ in chloroplasts. It seems that proline may enhance the production of photosynthetic pigments of the tolerant *M. oleifera* under salt stress.

As for the impact of inoculation with VAM, the inoculated seedlings with VAM has induced the highest value in chlorophyll a (73.56 and 79.44 mg/100g for first and second season, respectively) and chlorophyll b (53.86 and 64.66mg/100g for first and second season, respectively) (Tables 9 and 10).

### Table 7. Total dry weight (g) of inoculated and uninoculated seedlings of *Moringa oleifera* Lam. with VAM, unfertilized and fertilized with RP under five levels of salinity

| Rock phosphate (ppm) | Salinity level | VAM inoculation | RP*S | RP | S | VAM inoculation | RP*S | RP | S |
|----------------------|---------------|----------------|------|----|---|----------------|------|----|---|
|                      |               |                |      |    |   |                |      |    |   |
|                      | C             | VAM            | RP   | S  |   | C              | VAM  |   |   |
| Rp$_1$               | S$_1$         | 11.741         | 9.251 | 10.496 | 11.897 | 10.148 | 11.023 |
|                      | S$_2$         | 6.886          | 18.873 | 12.880 | 6.829 | 19.298 | 13.264 |
|                      | S$_3$         | 14.710         | 6.247 | 10.479 | 15.135 | 7.025 | 11.080 |
|                      | S$_4$         | 4.201          | 15.283 | 9.742 | 4.235 | 16.379 | 10.307 |
|                      | S$_5$         | 12.863         | 5.429 | 9.146 | 13.293 | 5.964 | 9.629 |
| Rp$_1$*VAM           | S$_1$         | 10.080         | 11.017 | 10.548 | 10.278 | 11.963 | 11.120 |
|                      | S$_2$         | 12.421         | 9.117 | 10.769 | 12.632 | 8.752 | 10.692 |
|                      | S$_3$         | 8.122          | 11.076 | 9.599 | 8.152 | 11.135 | 9.644 |
|                      | S$_4$         | 13.885         | 9.155 | 11.520 | 14.295 | 9.209 | 11.752 |
|                      | S$_5$         | 1.202          | 3.049 | 2.126 | 1.156 | 3.045 | 2.101 |
| Rp$_2$*VAM           | S$_1$         | 7.788          | 7.291 | 7.539 | 7.935 | 7.221 | 7.578 |
|                      | S$_2$         | 3.272          | 2.687 | 2.980 | 1.953 | 3.302 | 2.628 |
|                      | S$_3$         | 14.542         | 11.457 | 13.000 | 13.653 | 12.402 | 13.028 |
|                      | S$_4$         | 14.385         | 26.335 | 16.756 | 15.629 | 28.649 | 17.555 |
|                      | S$_5$         | 4.509          | 9.065 | 6.787 | 3.959 | 10.114 | 7.037 |
| Rp$_3$*VAM           | S$_1$         | 8.608          | 14.494 | 11.551 | 8.558 | 15.521 | 12.039 |
|                      | S$_2$         | 7.741          | 5.965 | 6.853 | 7.636 | 6.260 | 6.948 |
|                      | S$_3$         | 4.884          | 11.098 | 7.991 | 4.872 | 11.816 | 8.344 |
| S*VAM                | S$_1$         | 8.596          | 6.906 | 7.751 | 9.012 | 7.143 | 8.077 |
|                      | S$_2$         | 2.644          | 5.499 | 4.072 | 2.558 | 5.930 | 4.244 |
|                      | S$_3$         | 2.573          | 3.171 | 2.872 | 2.659 | 3.355 | 3.007 |
| VAM                  | S$_1$         | 8.825          | 10.934 | 8.924 | 11.568 | 8.05  | 4.407 |
| LSD at               | Rp$_1$        | S= 0.0.321     | VAM = 1.457 | Rp$_2$ | VAM = 1.814 | S= 0.73254 | 4.7514 |
Table 8. Shoot root ratio of inoculated and uninoculated seedlings of *Moringa oleifera* Lam. with VAM, unfertilized and fertilized with RP under five levels of salinity

| Rock phosphate RP | Salinity level (ppm) | VAM inoculation | VAM | RP*S | RP | S | VAM inoculation | RP*S | RP | S |
|------------------|---------------------|----------------|------|------|----|---|----------------|------|----|---|
|                  |                     |                |      |      |    |   |                |      |    |   |
|                  | S1                  | 0.185          | 0.450| 0.289|    |    | 0.156          | 0.533| 0.304|
|                  | S2                  | 0.417          | 0.242| 0.285|    |    | 0.352          | 0.287| 0.303|
| 0.0g             | S3                  | 0.062          | 0.827| 0.213|    |    | 0.053          | 0.979| 0.236|
|                  | S4                  | 0.232          | 0.209| 0.214|    |    | 0.196          | 0.247| 0.237|
|                  | S5                  | 0.033          | 0.448| 0.129|    |    | 0.028          | 0.529| 0.144|
|                  | **RP*S**            | 0.133          | 0.331| 0.229|    |    | 0.113          | 0.392| 0.247|
|                  | **S**               | 0.376          | 1.101| 0.611|    |    | 0.348          | 0.941| 0.541|
|                  | **RP**              | 0.825          | 0.279| 0.464|    |    | 0.765          | 0.239| 0.417|
| 1.0g             | S3                  | 0.129          | 0.282| 0.185|    |    | 0.119          | 0.241| 0.164|
|                  | S4                  | 0.363          | 0.312| 0.342|    |    | 0.336          | 0.267| 0.307|
|                  | S5                  | 0.000          | 0.720| 0.720|    |    | 0.000          | 0.618| 0.618|
|                  | **RP*S**            | 0.339          | 0.468| 0.398|    |    | 0.314          | 0.400| 0.353|
|                  | **S**               | 0.290          | 0.306| 0.297|    |    | 0.167          | 0.363| 0.253|
|                  | **RP**              | 0.290          | 0.306| 0.297|    |    | 0.167          | 0.363| 0.253|
| 2.0g             | S3                  | 0.150          | 0.494| 0.324|    |    | 0.203          | 0.466| 0.336|
|                  | S4                  | 0.986          | 0.691| 0.779|    |    | 0.678          | 0.819| 0.777|
|                  | S5                  | 0.000          | 0.244| 0.244|    |    | 0.000          | 0.289| 0.289|
|                  | **RP*S**            | 0.322          | 0.399| 0.52 |    |    | 0.266          | 0.443| 0.375|
|                  | **S**               | 0.281          | 0.530| 0.379| 0.379| 0.218| 0.547| 0.347|
|                  | **RP**              | 0.543          | 0.275| 0.347| 0.347| 0.483| 0.308| 0.354|
| S*VAM            | S3                  | 0.112          | 0.478| 0.250| 0.250| 0.123| 0.472| 0.254|
|                  | S4                  | 0.471          | 0.348| 0.385| 0.385| 0.369| 0.400| 0.390|
|                  | S5                  | 0.033          | 0.409| 0.212| 0.212| 0.028| 0.436| 0.222|
|                  | VAM                 | 0.247          | 0.390|      | 0.52 | 0.266| 0.443| 0.375|
|                  | **LSD at 0.05**     | **RP: 0.00235**| **S: 0.00241**| **VAM = 0.06632**| **RP = 0.00246**| **S = 0.00415**| **AM = 0.14114**| **RP*VAM = 0.056741**| **RP*S*VAM = 0.00233**| **0.05**| **RF*VAM = 0.05647**| **RP*VAM = 0.056741**| **RP*S*VAM = 0.00888**|

Table 9. Cholorophyll a (mg/100g) of inoculated and uninoculated seedlings of *Moringa oleifera* Lam. with VAM, unfertilized and fertilized with RP under five levels of salinity

| Rock phosphate RP | Salinity level (ppm) | VAM inoculation | VAM | RP*S | RP | S | VAM inoculation | RP*S | RP | S |
|------------------|---------------------|----------------|------|------|----|---|----------------|------|----|---|
|                  |                     |                |      |      |    |   |                |      |    |   |
|                  | S1                  | 70.36          | 78.2 | 74.28 |    |    | 74.58          | 84.46| 79.52|
|                  | S2                  | 65.37          | 74.57| 69.97 |    |    | 69.29          | 80.54| 74.915|
| 0.0g             | S3                  | 64.82          | 73.02| 68.92 |    |    | 68.71          | 78.86| 73.785|
|                  | S4                  | 62.29          | 70.49| 66.39 |    |    | 66.03          | 76.13| 71.08|
|                  | S5                  | 59.56          | 67.76| 63.66 |    |    | 63.13          | 73.18| 68.155|
|                  | **RP*S**            | 64.48          | 72.81| 68.64 | 68.64| 0.215| 0.416| 0.05|
|                  | **S**               | 68.34          | 76.54| 72.44 |    | 72.44| 82.66| 77.55|
|                  | **RP**              | 66.63          | 72.83| 69.73 |    | 70.63| 78.66| 74.645|
| 1.0g             | S3                  | 66.1           | 72.3 | 69.2  |    |    | 70.07          | 78.08| 74.075|
|                  | S4                  | 63.47          | 68.67| 66.07 |    |    | 67.28          | 74.16| 70.72|
|                  | S5                  | 60.91          | 69.11| 65.01 |    |    | 64.56          | 74.64| 69.6 |
### Table 9. Chlorophyll a (mg/ 100g) of inoculated and uninoculated seedlings of *Moringa oleifera* Lam. with VAM, unfertilized and fertilized with RP under five levels of salinity

| Rock phosphate | Salinity level (ppm) | VAM inoculation | RP*S | RP | S | VAM inoculation | RP*S | RP | S |
|----------------|----------------------|-----------------|------|----|---|-----------------|------|----|---|
| Rp₂*VAM        |                      |                 |      |    |   |                 |      |    |   |
| S1             | 65.09                | 71.89           | 68.49| 69 | 77.64 | 73.32           |     |
| Rp₃            |                      |                 |      |    |   |                 |      |    |   |
| S1             | 71.78                | 79.98           | 75.88| 76.09 | 86.38 | 81.235          |     |
| Rp₂*VAM        |                      |                 |      |    |   |                 |      |    |   |
| S1             | 71.06                | 78.24           | 74.2 | 74.37 | 84.5  | 79.44           |     |
| Rp₃            |                      |                 |      |    |   |                 |      |    |   |
| S2             | 67.07                | 74.94           | 71.01| 71.1 | 80.94 | 76.02           |     |
| S1             | 65.55                | 74.08           | 70.31| 70.54 | 80    | 75.27           |     |
| Rp₂*VAM        |                      |                 |      |    |   |                 |      |    |   |
| S3             | 63.89                | 71.09           | 67.49| 67.72 | 76.77 | 72.25           |     |
| Rp₃            |                      |                 |      |    |   |                 |      |    |   |
| S4             | 40.16                | 69.46           | 54.81| 42.56 | 75.02 | 58.79           |     |
| Rp₂*VAM        |                      |                 |      |    |   |                 |      |    |   |
| S5             | 61.56                | 73.56           | 65.26| 79.44 |     |     |                 |
| LSD at         |                      |                 |      |    |   |                 |      |    |   |
| RP:           |                      |                 |      |    |   |                 |      |    |   |
| Rp₂*VAM        |                      |                 |      |    |   |                 |      |    |   |
| S= 3.026       | 4.523               | 4.0025          |     |     |     |                 |
| RP=           |                      |                 |      |    |   |                 |      |    |   |
| Rp₂*VAM        |                      |                 |      |    |   |                 |      |    |   |
| S= 3.0798      | 4.555               | 4.0369          |     |     |     |                 |

### Table 10. Chlorophyll b (mg/ 100g) of inoculated and uninoculated seedlings of *Moringa oleifera* Lam. with VAM, unfertilized and fertilized with RP under five levels of salinity

| Rock phosphate | Salinity level (ppm) | VAM inoculation | RP*S | RP | S | VAM inoculation | RP*S | RP | S |
|----------------|----------------------|-----------------|------|----|---|-----------------|------|----|---|
| Rp₁            |                      |                 |      |    |   |                 |      |    |   |
| Rp₂*VAM        |                      |                 |      |    |   |                 |      |    |   |
| S= 3.0798      | 4.555               | 4.0369          |     |     |     |                 |
| Rp₃            |                      |                 |      |    |   |                 |      |    |   |
| Rp₂*VAM        |                      |                 |      |    |   |                 |      |    |   |
| S= 3.0798      | 4.555               | 4.0369          |     |     |     |                 |

### Notes:
- LSD at: Least Significant Difference at 0.05 level.
4.2. Proline content (g/100g).

There is a significant increase in proline accumulation in both seasons with the highest rate of increase in salinity. Proline is increased significantly with the increasing in the concentration of salinity at the fifth level of NaCl (S5) in both seasons (13.54 and 15.90g/100g, for first and second season, respectively) (Tables, 11).

As for the effect of inoculation with VAM, the inoculated seedlings with VAM induced the highest value in proline content in the first and second seasons (11.18 and 13.73 g/100g, for first and second season, respectively) (Tables, 11).

4.3 Mineral contents (N, P, K and Na) of leaves.

Significant depressions were obtained in potassium concentration as a result of growing seedlings of Moringa oleifera under salinity condition in both seasons (Table 13), while nitrogen increased significantly only with the third level of salinity S3. Phosphorous concentration increased was significantly under the fourth level of salinity S4 (Tables 11 and 12). Regardless, the effect of salinity and RP application, the inoculated seedlings with VAM fungus displayed the highest values in N (3.42 and 3.86% for first and second season, respectively), K (2.17 and 2.04% for first and second season, respectively) and P (0.70 and 0.69% for first and second season, respectively). (Tables, 11, 12 and 13).

Table 11. Proline content (g/ 100g) of inoculated and uninoculated seedlings of Moringa oleifera Lam. with VAM, unfertilized and fertilized with RP under five levels of salinity

| Rock phosphate | Salinity level (ppm) | VAM inoculation | RP*S | RP | S | VAM inoculation | RP*S | RP | S |
|---------------|----------------------|----------------|------|----|---|----------------|------|----|---|
|               |                      |                |      |    |   |                |      |    |   |
| Rp1 0.0g S1  | C                    | 1.31           | 5.38 | 3.35 | 1.28 | 5.50 | 3.39 |
| Rp2 1.0g S2  |                      | 3.42           | 7.28 | 5.35 | 3.56 | 7.45 | 5.40 |
| Rp3 2.0g S3  |                      | 3.43           | 9.47 | 6.45 | 3.36 | 9.85 | 6.61 |
| Rp4 3.0g S4  |                      | 4.45           | 9.74 | 7.10 | 4.32 | 10.13| 7.23 |
| Rp5 4.0g S5  |                      | 12.37          | 18.96| 15.67| 12.00| 19.72| 15.86|
| Rp1*VAM S1  |                      | 5.00           | 10.17| 7.58 | 4.86 | 10.53| 7.70 |
| Rp2*VAM S2  |                      | 1.61           | 6.45 | 4.03 | 1.56 | 8.00 | 4.78 |
| Rp3*VAM S3  |                      | 4.62           | 9.68 | 7.15 | 4.48 | 12.00| 8.24 |
| Rp4*VAM S4  |                      | 5.17           | 10.89| 8.03 | 4.60 | 13.50| 9.05 |
| Rp5*VAM S5  |                      | 5.45           | 10.47| 8.10 | 4.85 | 13.32| 9.09 |
| S*VAM S1    |                      | 3.37           | 11.34| 7.36 | 3.10 | 14.07| 8.58 |
| S*VAM S2    |                      | 1.11           | 6.77 | 3.94 | 1.23 | 8.26 | 4.75 |
| S*VAM S3    |                      | 3.02           | 9.78 | 6.40 | 3.35 | 13.69| 8.52 |
| S*VAM S4    |                      | 3.63           | 11.63| 7.63 | 4.03 | 16.28| 10.16|
| S*VAM S5    |                      | 3.77           | 11.89| 7.83 | 4.18 | 16.65| 10.42|
| VAM          |                      | 4.48           | 12.02| 8.25 | 4.97 | 16.59| 10.78|
| VAM          |                      | 1.34           | 6.20 |      | 3.77 | 13.6| 7.25 |
| VAM          |                      | 3.69           | 8.91 |      | 6.30 | 11.05| 7.39 |
| VAM          |                      | 4.08           | 10.66|      | 7.37 | 13.21| 8.60 |
| VAM          |                      | 4.56           | 10.79|      | 7.67 | 13.37| 8.91 |
| VAM          |                      | 7.75           | 19.32|      | 13.54| 23.76| 15.90|
| LSD at       |                      |                |      |      | 4.28 | 11.18| 4.31 |
| Rp: 1.0365   | S= 3.652             |                |      |      |      |      |      |
| VAM = 5.654  | RP*S*VAM= 5.369      |                |      |      |      |      |      |
| 0.05         | VAM = 5.847          | RP*S*VAM= 5.254 |      |      |      |      |      |
Table 12. Nitrogen (N) content (%) of inoculated and uninoculated seedlings of *Moringa oleifera* Lam. with VAM, unfertilized and fertilized with RP under five levels of salinity

| Rock phosphate (ppm) | Salinity level | VAM inoculation | RP*S | RP | S | VAM inoculation | RP*S | RP | S |
|----------------------|----------------|-----------------|------|----|---|-----------------|------|----|---|
|                      |                | C               | VAM  |    |    | C               | VAM  |    |    |
| Rp1 0.0g             | S1             | 1.92            | 2.10 | 2.01 | 1.80 | 1.97 | 1.89 |
|                     | S2             | 2.21            | 2.66 | 2.44 | 2.08 | 2.50 | 2.29 |
|                     | S3             | 2.21            | 2.31 | 2.26 | 2.08 | 2.17 | 2.13 |
|                     | S4             | 1.77            | 3.25 | 2.51 | 1.66 | 3.06 | 2.36 |
|                     | S5             | 1.44            | 2.31 | 1.88 | 1.35 | 2.17 | 1.76 |
| Rp1*VAM             | S1             | 1.91            | 2.53 | 2.22 | 1.79 | 2.37 | 2.08 |
|                     | S2             | 1.22            | 2.93 | 2.08 | 1.26 | 2.99 | 2.13 |
|                     | S3             | 1.66            | 2.78 | 2.22 | 1.71 | 2.83 | 2.27 |
|                     | S4             | 1.66            | 2.96 | 2.31 | 1.71 | 3.02 | 2.37 |
|                     | S5             | 1.22            | 2.85 | 2.04 | 1.26 | 2.91 | 2.09 |
| Rp2*VAM             | S1             | 1.42            | 2.90 | 2.16 | 1.46 | 2.96 | 2.21 |
|                     | S2             | 1.88            | 2.21 | 2.05 | 2.54 | 2.98 | 2.76 |
|                     | S3             | 1.63            | 3.41 | 2.52 | 2.20 | 3.98 | 3.09 |
|                     | S4             | 2.21            | 4.34 | 3.28 | 2.98 | 4.19 | 3.59 |
|                     | S5             | 1.33            | 2.98 | 2.16 | 1.37 | 3.04 | 2.21 |
| Rp3*VAM             | S1             | 1.79            | 3.42 | 2.61 | 2.42 | 3.86 | 3.14 |
|                     | S2             | 1.67            | 2.41 | 2.04 | 1.87 | 2.65 | 2.26 |
|                     | S3             | 1.83            | 2.95 | 2.39 | 2.00 | 3.10 | 2.55 |
|                     | S4             | 1.78            | 2.99 | 2.39 | 1.98 | 3.09 | 2.54 |
|                     | S5             | 0.89            | 2.75 | 1.82 | 0.87 | 3.09 | 1.98 |
| VAM                 | S1             | 1.67            | 2.41 | 2.04 | 1.87 | 2.65 | 2.26 |
|                     | S2             | 1.83            | 2.95 | 2.39 | 2.00 | 3.10 | 2.55 |
|                     | S3             | 1.78            | 2.99 | 2.39 | 1.98 | 3.09 | 2.54 |
|                     | S4             | 1.78            | 2.99 | 2.39 | 1.98 | 3.09 | 2.54 |
|                     | S5             | 0.89            | 2.75 | 1.82 | 0.87 | 3.09 | 1.98 |
| LSD at RP= ------- | S= 0.0942      | VAM = 1.0214    | RP= ------- | S=0.0632 | VAM = 1.0547 |
| 0.05                | Rp1*VAM= 0.3478 | VAM=1.0214      | Rp2*VAM= 0.3965 |

Table 13. Phosphorus (P) content (%) of inoculated and uninoculated seedlings of *Moringa oleifera* Lam. with VAM, unfertilized and fertilized with RP under five levels of salinity

| Rock phosphate (ppm) | Salinity level | VAM inoculation | RP*S | RP | S | VAM inoculation | RP*S | RP | S |
|----------------------|----------------|-----------------|------|----|---|-----------------|------|----|---|
|                      |                | C               | VAM  |    |    | C               | VAM  |    |    |
| Rp1 0.0g             | S1             | 0.25            | 0.39 | 0.32 | 0.25 | 0.37 | 0.31 |
|                     | S2             | 0.19            | 0.46 | 0.33 | 0.22 | 0.44 | 0.33 |
|                     | S3             | 0.19            | 0.45 | 0.32 | 0.19 | 0.43 | 0.31 |
|                     | S4             | 0.29            | 0.54 | 0.42 | 0.28 | 0.52 | 0.40 |
|                     | S5             | 0.29            | 0.36 | 0.33 | 0.28 | 0.35 | 0.32 |
| Rp1*VAM             | S1             | 0.24            | 0.44 | 0.34 | 0.24 | 0.42 | 0.33 |
|                     | S2             | 0.27            | 0.39 | 0.33 | 0.26 | 0.37 | 0.32 |
|                     | S3             | 0.20            | 0.52 | 0.36 | 0.20 | 0.50 | 0.35 |
|                     | S4             | 0.17            | 0.45 | 0.31 | 0.17 | 0.43 | 0.30 |
|                     | S5             | 0.19            | 0.38 | 0.29 | 0.19 | 0.36 | 0.28 |
| Rp2*VAM             | S1             | 0.24            | 0.34 | 0.29 | 0.24 | 0.33 | 0.29 |
Upon the significant interaction among the three factors studied. However, the highest N content % was obtained in the inoculated seedlings which applied with the third level of RP and treated with S3 level of salinity (4.34 and 4.19% for first and second season, respectively), the highest K content % was obtained in the inoculated seedlings which applied with the third level of RP and treated with S3 level of salinity in the (2.51 and 2.36% for first and second season, respectively) (Tables, 11 and 13), but the highest values of P were obtained at the fourth level of salinity S4 (1.46 and 1.40% for first and second season, respectively), the highest K content % was obtained in the inoculated seedlings which applied with the third level of salinity in the (4.34 and 4.19% for first and second season, respectively) (Table 13).

Data showed in Table 14 that Na content increased with increases in Na Cl levels, reaching the highest value (0.55 % and 0.53 %) in the first and second season respectively for Na Cl 171.1 mM, while, Na content decreased with increases in RP levels, reaching the lowest value (0.0.46 % and 0.45 %) in the first and second season respectively for RP3(2g/kg soil). (Table 14).

These data are in accordance with those Ashraf and Orooj, 2006) and (Tabatabaie and Nazari, 2007). However, the relation between salinity and minerals nutrition of plants are very complex (Grattan and Grieve, 1999).

**DISCUSSION**

The obtained results showed that the inoculation with VAM and addition of RP led to enhance the growth significantly, in terms of S, SH, SRR, RDW, SDW and TDW and minerals of the leaves of *M. oleifera* (N%, P% and K%) compared with the uninoculated ones. This may owing to the ability of mycorrhiza to increase root surface area to uptake mineral contents and make phosphorus absorbable by plant roots. This result was in agreement with the finding of Pagano et al. (2010) who reported that VAM colonization was significantly higher with the inoculated seedlings versus non-inoculated ones (control) and Tazisong et al. (2015) who said that Phosphatases are responsible for the hydrolysis of a range of organic P compounds and provide mineral phosphate to the plant. Furthermore, Matias et al. (2009) reported that the intensity of VAM colonization was also stimulated by plant growth.

It is worth noting that there is a significant decreasing of growth parameters with increasing in salinity level. These results in accordance with findings of Wang, et al., (2009); Ayse Sen and Sema Alikamanoglou (2011) and Omneya, et al. (2018).

Our results show that the increase of available P in rhizosphere was clearly related to the inoculation with the VAM treatment. Noteworthy, the increase in available P in the rhizosphere was clearly affected by VAM colonization in host plants. These findings are in match with Soon-Jae, et al. (2020).

The uptake of N and P was higher in VAM seedlings, and as the salinity increased, the trend showed a decline but had a clear upturn as the salinity stress increased to a high level (Dastogeer, et al., 2020). A number of reports emphasized the important role of mycorrhiza in
salinity tolerance of plants due to reduced proline accumulation in the leaves of salinity affected plants (Heikham et al., 2019). In the present study, we found a significant increase in proline accumulation in both season with the highest level in salinity. These results are in accordance with those obtained by Szabados and Savoure (2009), yet proline content was decreased in VAM+RP treatment.

The increased chlorophyll content due to VAM inoculation under normal as well as salinity stress corroborates the reports of Arora et al. (2013) in lettuce, Alqarawi et al. (2014) in Tamarix aphylla and Abd_Allah et al. (2015) in Sesbania sesban. Recently, in salt-stressed Brassica juncea, Ahmad et al. (2015)

Table 14. Potassium (K) content (%) of inoculated and uninoculated seedlings of Moringa oleifera Lam. with VAM, unfertilized and fertilized with RP under five levels of salinity

| Rock phosphate (ppm) | Salinity level | VAM inoculation | RP*S | VAM inoculation | RP*S |
|----------------------|---------------|----------------|-------|----------------|-------|
| Rp1 0.0g             | C VAM         | 1.96 1.99      | 1.98  | 1.82 1.87      | 1.85  |
|                      | S1            | 1.84 1.87      | 1.86  | 1.71 1.76      | 1.74  |
|                      | S2            | 1.65 1.86      | 1.76  | 1.53 1.75      | 1.64  |
|                      | S3            | 1.86 1.75      | 1.81  | 1.73 1.65      | 1.69  |
|                      | S4            | 1.62 1.71      | 1.67  | 1.51 1.61      | 1.56  |
|                      | S5            | 1.79 1.84      | 1.81  | 1.66 1.73      | 1.69  |
| Rp1*VAM              |               |                |       |                |       |
|                      | S1            | 2.04 2.10      | 2.07  | 1.90 1.97      | 1.94  |
|                      | S2            | 1.86 1.87      | 1.87  | 1.73 1.76      | 1.75  |
|                      | S3            | 1.81 1.90      | 1.86  | 1.68 1.79      | 1.74  |
|                      | S4            | 1.93 1.93      | 1.93  | 1.79 1.81      | 1.80  |
|                      | S5            | 1.63 1.68      | 1.66  | 1.52 1.58      | 1.55  |
| Rp2 1.0g             |                |                |       |                |       |
|                      | S1            | 1.84 2.22      | 2.03  | 1.71 2.09      | 1.90  |
|                      | S2            | 1.81 2.34      | 2.08  | 1.68 2.20      | 1.94  |
|                      | S3            | 1.81 2.51      | 2.16  | 1.68 2.36      | 2.02  |
|                      | S4            | 1.82 1.95      | 1.89  | 1.69 1.83      | 1.76  |
|                      | S5            | 0.00 1.82      | 0.91  | 0.00 1.71      | 0.86  |
| Rp2*VAM              |                |                |       |                |       |
|                      | S1            | 1.84 1.90      | 1.88  | 1.72 1.78      | 1.75  |
|                      | S2            | 1.81 2.22      | 2.03  | 1.71 2.09      | 1.90  |
|                      | S3            | 1.81 2.34      | 2.08  | 1.68 2.20      | 1.94  |
|                      | S4            | 1.82 1.95      | 1.89  | 1.69 1.83      | 1.76  |
|                      | S5            | 0.00 1.82      | 0.91  | 0.00 1.71      | 0.86  |
| Rp3 2.0g             |                |                |       |                |       |
|                      | S1            | 1.95 2.10      | 2.03  | 1.81 1.98      | 1.89  |
|                      | S2            | 1.84 2.03      | 1.93  | 1.71 1.91      | 1.81  |
|                      | S3            | 1.76 2.09      | 1.92  | 1.63 1.97      | 1.80  |
|                      | S4            | 1.87 1.88      | 1.87  | 1.74 1.76      | 1.75  |
|                      | S5            | 1.08 1.74      | 1.41  | 1.01 1.63      | 1.32  |
| S*VAM                |                |                |       |                |       |
|                      | S1            | 1.70 1.97      | 1.58  | 1.58 1.85      |       |
|                      | S2            | 1.46 2.17      | 1.81  | 1.35 2.04      | 1.70  |
|                      | S3            | 1.95 2.10      | 2.03  | 1.81 1.98      | 1.89  |
|                      | S4            | 1.84 2.03      | 1.93  | 1.71 1.91      | 1.81  |
|                      | S5            | 1.76 2.09      | 1.92  | 1.63 1.97      | 1.80  |
|                      | S6            | 1.87 1.88      | 1.87  | 1.74 1.76      | 1.75  |
|                      | S7            | 1.08 1.74      | 1.41  | 1.01 1.63      | 1.32  |
| VAM                  |                |                |       |                |       |
|                      | S1            | 1.70 1.97      | 1.58  | 1.58 1.85      |       |
|                      | S2            | 1.46 2.17      | 1.81  | 1.35 2.04      | 1.70  |
|                      | S3            | 1.95 2.10      | 2.03  | 1.81 1.98      | 1.89  |
|                      | S4            | 1.84 2.03      | 1.93  | 1.71 1.91      | 1.81  |
|                      | S5            | 1.76 2.09      | 1.92  | 1.63 1.97      | 1.80  |
|                      | S6            | 1.87 1.88      | 1.87  | 1.74 1.76      | 1.75  |
|                      | S7            | 1.08 1.74      | 1.41  | 1.01 1.63      | 1.32  |

Our results indicated that, irrespective of salinity treatments, studied mineral contents increased with the inoculation with VAM fungus were counteracted partially or completely the adverse effect of salinity as it increased the concentrations of N, P, and K in the same time it decreased the absorption of Na and M. oleifera leaves compared with the corresponding salinity levels. (Omneya. et al., 2018.) The largest increases in plants nutrient uptake (N, P and K) were observed with the VAM+RP treatment. Similar results were obtained by Ortas and Ustuner (2014). Thus phosphorus may alleviate the harmful effect of salinity and may boost salinity tolerance (Amel, et al., 2019 and Matthew and Olubukola 2018).
In the present study, we found a significant increase in proline accumulation in both seasons under the highest salinity level, especially that supplemented by the aid of symbiotic agents such as mycorrhiza as it is in our study and also Frankia (El-Settawy and Ei-Gamal, 2009). In addition, *M. oleifera* plants can synthesize proline, they have been shown to take up exogenous proline and accumulate it Omneya, *et al.*, (2018). Synthesis of amino acids is very important, notably, proline, glutamic (*Flowers et al.*, 1977 and Fayek. *et al.*, 2010 and Dastogeer, *et al.*, 2020) and glycine betaine (*Subbarao and Parvaize* 2001) to create cellular osmotic balance, it is well known that the amino acis , proline is increased considerably under salinity stress and it could reached 200 fold that of plants in normal conditions (*Elefin et al.*, 2019 and Xie, *et al.*, 2020)

Finally, our results support the significant roles of rock phosphate in the alleviation of salt stress and enhancing soil quality for better symbiosis efficiency and yield obtained of *M. oleifera*.

### CONCLUSIONS AND RECOMMENDATIONS

The present study concluded that (*M. oleifera* Lam.) could tolerate salt concentration up to 171.1 mM in presence of mycorrhiza. Negative relationship was shown between salt stress degree and plant growth parameters, expressed as SH, RDW, SRR, SDW and RDW which decreased as the salt concentration increased.

Therefore, is recommended, however, to inoculate the seedlings with VAM and rock-phosphate application RP2 (1g/kg soil) to enhance its growth and to gain tolerance against salinity stress.
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تأثير فطر الميكوريزا الحويصلية الشجيرية وإضافة صخر الفوسفات على نمو والكتلة الحيوية لشتلات المورينجا أوليفرا تحت الإجهاد الملحي

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الملخص العربي

تأثير فطر الميكوريزا الحويصلية الشجيرية وإضافة صخر الفوسفات على نمو والكتلة الحيوية لشتلات المورينجا أوليفرا تحت الإجهاد الملحي

الملوحة عامل إجهاد بيئي خطير يؤثر بشدة على نمو النباتات وتكشفها، وتسبب ملوحة التربة غالبا تأخير الإنباهة

النباتية في كلا الحالتين الطبيعية والزراعية. فطر الميكوريزا الحويصلية الشجيرية المتكافئة يمكن أن يقلل من الإجهاد النباتي الناتج عن الملوحة وذلك عن طريق زيادة درجة التحمل للملوحة.

أجري هذا البحث في موسمي نمو بدأ من يونيه 2017 حتى مايو 2018 وتم تكرار التجربة في نفس المواعيد في الموسم التالي وذلك بمشتل قسم الغابات وتكنولوجيا الأخشاب بمحطة البحوث الزراعية - كلية الزراعة - جامعة الإسكندرية

منطقة أسيوئس وكذلك معامل قسم الغابات وتكنولوجيا الأخشاب بالكلية.

أوضح النتائج المتحصل عليها أن التلقح بواسطة VAM وإضافة صخر الفوسفات أدت إلى تشجيع جوهري للنمو معاً منها في الحيوية وارتفاع النبات والوزن الجاف للمجموع الخضري والوزن الجاف للجذر ونسبة الساق إلى الجذر والوزن الجاف الكلي والعناصر المعدنية في الأوراق N, P, K

واختصار في تركيز الصوديوم في الأوراق مقارنة بالشتلات غير الملوحة بالميكوريزا. أثرت معاملات كورةيد الصوديوم على محتوى الأوراق من الكلوروفيل حيث انخفض تركيز كلوروفيل أ وب في الأوراق في كل موصى التنمو.

أوضحت النتائج أن أعلى تركيز للعناصر المعدنية في الأوراق كانت في حالة المعاملة الثانية للشتلات بالميكوريزا وإضافة صخر الفوسفات.

كما أظهرت النتائج أن أعلى تركيز للبروتين في شتلات المورينجا الملحة بالميكوريزا في كلا موصى التنمو. كما انصح من التجربة أن شتلات المورينجا يمكّنها أن تحمل تركيز ملحي عالي يصل إلى 171 ميليمول في وجود الميكوريزا.

نصح بأن يتم تلقيح شتلات المورينجا بفطر الميكوريزا وإضافة صخر الفوسفات بمعدل (1جم/كجم تربة). وذلك لزيادة سرعة النمو وأكسب الشتلات تحمل للإجهاد الملحي.

الكلمات الدالة: ملوحة- بروتين- ميكوريزا- مورينجا- صخر الفوسفات.