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 annuals, 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

DOI: 10.21608/asejaiqjsae.2021.167455

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Received March 21, 2021, Accepted, April 28, 2021.

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_2O and oxygen is superoxide dismutase (SOD) (Alscher *et al*., 2002). The main enzyme involved in H_2O_2 scavenging is also catalase, which decomposes H_2O_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_2O_2 (Van Breusegem *et al*., 2001).

Moringa oleifera in Pakistan named as sohanjna is a miracle tree having tremendous uses like phytopesticides, 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 mycorrhizial (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 productivityunder 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 fingus, *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 tratments 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 Yamashta, 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
HCO_3^-	2.4
SO_4^{--}	26.4
CO_3^{--}	-----
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 inculated 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.

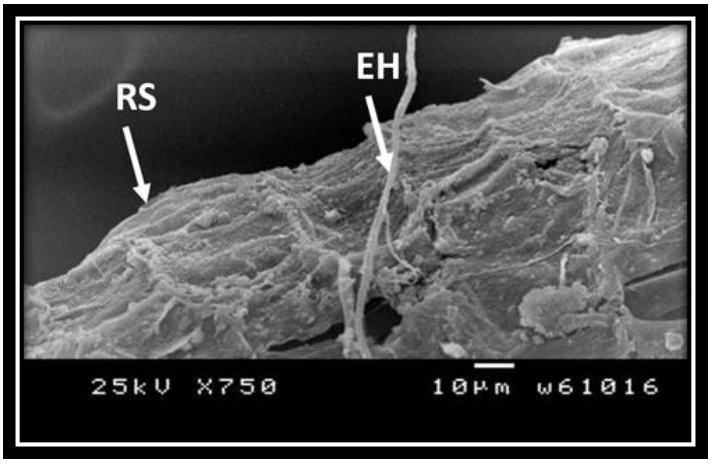

Fig. 1. Scanning electron micrograph indicates root surface (RS) penetrated by extrametrical hyphae of VAM fungus (EH)

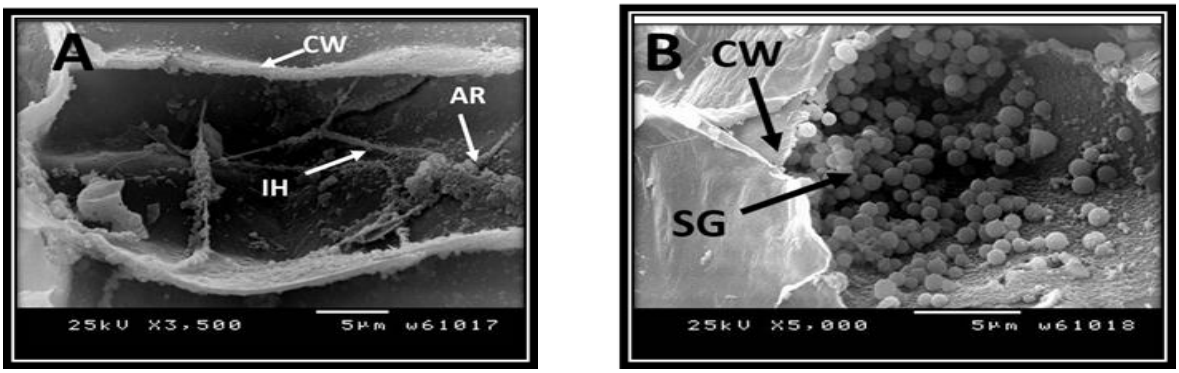

Fig. 2. (A) Scanning electron micrograph (SEM) indicates feeder root of *Moringa oleifera* contained Arbscules of *Glomus fasciculatum*. IH: Internal hyhpae, Ar: Arbscule and CW:Cell wall. (B) Mature parenchymateous cells with starch granules (SG) in the cortex of feeder root cell

2.1. Survival (S) (%)

Regardless of the impact of salinity and inoculation with VAM, there is non singnificant 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 S_5 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).

Table 3. Survival (%) of inoculated and uninoculated seedlings of *Moringa oleifera* Lam. with VAM, unfertilized and fertilized with RP under five levels of salinity

		First season					Second season				
Rock phosphate RP	**Salinity level (ppm)**	**VAM inoculation**		**RP*S**	**RP**	**S**	**VAM inoculation**		**RP*S**	**RP**	**S**
		C	**VAM**				**C**	**VAM**			
	S_1	100	100	100			100	100	100		
Rp_1	S_2	100	100	100			100	100	100		
0.0g	S_3	100	100	100			100	100	100		
	S_4	75	100	87.50			100	100	100		
	S_5	75	100	87.50			75	100	87.50		
RP_1*VAM		90.00	100		95.0		95.00	100		97.50	
	S_1	100	100	100			100	100	100		
RP_2	S_2	100	100	100			100	100	100		
1.0g	S_3	100	100	100			100	100	100		
	S_4	50	100	75			75	100	87.50		
	S_5	50	75	62.50			75	100	87.50		
Rp_2*VAM		90.00	95.00		92.50		95	100		97.50	
	S_1	100	100	100			100	100	100		
Rp_3	S_2	100	100	100			100	100	100		
2.0g	S_3	100	100	100			100	100	100		
	S_4	75	100	87.50			75.00	100	87.50		
	S_5	0.00	75	37.50			0.00	75	37.50		
Rp_3*VAM		75.	95.00		85.0		75	95.00		85.00	
	S_1	100	100			100	100	100			100
	S_2	100	100			100	100	100			100
S*VAM	S_3	100	100			100	100	100			100
	S_4	83.33	100			91.67	91.67	100			95.83
	S_5	41.67	83.33			62.50	50.00	91.67			70.83
VAM		85.00	96.67				88.33	98.33			
LSD at 0.05	RP= ------ S= 6.52 RP*S*= 2.0523			VAM = 9.62 RP*S*VAM= 13.54			RP= ----- S= 5.075 RP*S*= -------		VAM = 9.88 RP*S*VAM = 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 S_1 in both seasons (36.1 and 47.9cm for first and second season, respectively), whilst the lowest value was found in those treated with S_5 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 RP_2 and applied with S_2 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 RP_2 and applied with S_2 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 RP_3 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

		First season					Second season				
Rock phosphate RP	**Salinity level (ppm)**	**VAM inoculation**		**RP*S**	**RP**	**S**	**VAM inoculation**		**RP*S**	**RP**	**S**
		C	**VAM**				**C**	**VAM**			
	S_1	29.4	37.7	33.6			38.5	47.1	42.8		
Rp_1	S_2	25.3	40.4	32.9			34.5	49.8	42.2		
0.0g	S_3	23.6	38.6	31.1			32.9	47.9	40.4		
	S_4	23.6	35.8	29.7			32.9	45.2	39.1		
	S_5	13.0	30.3	21.7			22.3	39.8	31.1		
RP_1*VAM		23.0	36.6		29.8		32.2	46.0		39.1	
	S_1	31.0	37.6	34.3			37.9	37.2	37.6		
RP_2	S_2	29.4	49.9	39.7			36.4	48.7	42.6		
1.0g	S_3	24.4	41.7	33.1			31.8	40.5	36.2		
	S_4	22.8	33.5	28.2			30.3	32.3	31.3		
	S_5	0.0	27.7	13.9			0.0	26.6	13.8		
Rp_2*VAM		21.5	38.1		29.8		27.3	37.1		32.3	
	S_1	29.4	36.8	33.1			20.6	31.6	26.1		
Rp_3	S_2	29.4	41.3	35.4			21.6	36.1	28.9		
2.0g	S_3	26.7	27.6	27.2			19.3	22.6	21.0		
	S_4	31.1	34.0	32.6			23.1	28.9	26.0		
	S_5	0.0	28.5	14.3			0.0	23.5	11.8		
Rp_3*VAM		23.3	33.6		28.5		16.9	28.5		22.7	
	S_1	29.9	37.4			33.7	32.3	38.6			35.5
	S_2	28.3	43.9			36.1	30.8	44.9			47.9
S*VAM	S_3	24.9	36.0			30.4	28.0	37.0			32.5
	S_4	25.8	34.4			30.1	28.8	35.5			32.1
	S_5	22.7	29.9			26.3	25.5	37.2			31.3
VAM		22.6	36.1				25.5	37.2			
LSD at 0.05	RP: ------- RP*VAM =4.37		S= 2.10	RP*S*VAM= 5.23	VAM = 2.46		RP: ------- VAM = 2.46 RP*S*VAM= 5.63			RP*VAM =4.41	S= 2.33

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

		First season					Second season				
Rock phosphate RP	**Salinity level (ppm)**	**VAM inoculation**		**RP*S**	**RP**	**S**	**VAM inoculation**		**RP*S**	**RP**	**S**
		C	**VAM**				**C**	**VAM**			
	S_1	1.831	2.871	2.351			1.607	3.528	2.5675		
Rp_1	S_2	2.026	3.683	2.8545			1.779	4.528	3.1535		
0.0g	S_3	0.86	2.827	1.8435			0.755	3.475	2.115		
	S_4	0.791	2.643	1.717			0.695	3.249	1.972		
	S_5	0.413	1.679	1.046			0.363	2.064	1.2135		
RP_1*VAM		1.1842	2.7406		1.9624		1.0398	3.3688		2.2043	
	S_1	3.391	4.777	4.084			3.262	4.242	3.752		
RP_2	S_2	3.672	2.416	3.044			3.532	2.145	2.8385		
1.0g	S_3	1.585	2.015	1.8			1.525	1.789	1.657		
	S_4	1.202	0.749	0.9755			1.156	0.665	0.9105		
	S_5	0	1.657	0.8285			0	1.472	0.736		
Rp_2*VAM		1.97	2.3228		2.1464		1.895	2.0626		1.9788	
	S_1	3.272	2.687	2.9795			1.953	3.302	2.6275		
Rp_3	S_2	3.095	6.695	4.895			2.789	8.229	5.509		
2.0g	S_3	1.875	6.327	4.101			2.639	6.19	4.4145		
	S_4	2.239	3.705	2.972			1.599	4.554	3.0765		
	S_5	0	1.267	0.6335			0	1.558	0.779		
Rp_3*VAM		2.0962	4.1362		3.1162		1.796	4.7666		3.2813	
	S_1	1.6988	2.067			1.8829	1.3644	2.2144			1.7894
	S_2	1.7196	2.3964			2.058	1.5856	2.7804			2.183
S*VAM	S_3	0.864	2.2338			1.5489	0.9838	2.2908			1.6373
	S_4	0.8464	1.4194			1.1329	0.69	1.6936			1.1918
	S_5	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 =0.1697						VAM = 1.76214			VAM*S =0.56841	
	RP*S*VAM= 3.233						RP*S*VAM= 4.463				

The inoculation with VAM has also brought about the highest SDW in both seasons (3.066 and 3.3993g), respectively, while uninoculated seedlings displayed the lowest value of SDW, since it was (1.750 and 1.576g for first and second season, respectively) (Table 5). Furthermore, the significant interaction between salinity level and VAM inoculation has revealed that the seedlings applied with S_2 and inoculated with VAM fungus induced the highest SDW (2.396 and 2.7804g for first and second season, respectively), followed by inoculated seedlings with VAM and applied with S_3 level (Table 5).

As for the significant triple interaction of the factors studied, it was found that the seedlings inoculated with VAM which applied with RP_3 amended with S_2 displayed the highest SDW in both seasons (6.695 and 8.229g for first and second season, respectively) (Table 5).

2.4. Root dry weight (RDW) (g)

There were significant effects of the salinity level. However, seedlings amended with S_3 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 Rp_3 and amended with S_2 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

		First season					Second season				
Rock phosphate RP	**Salinity level (ppm)**	**VAM inoculation**		**RP*S**	**RP**	**S**	**VAM inoculation**		**RP*S**	**RP**	**S**
		C	**VAM**				**C**	**VAM**			
	S_1	9.91	6.38	8.145			10.29	6.62	8.455		
Rp_1	S_2	4.86	15.19	10.025			5.05	15.77	10.41		
0.0g	S_3	13.85	3.42	8.635			14.38	3.55	8.965		
	S_4	3.41	12.64	8.025			3.54	13.13	8.335		
	S_5	12.45	3.75	8.1			12.93	3.9	8.415		
RP_1*VAM		8.896	8.276		8.586		9.238	8.594		8.916	
	S_1	9.03	4.34	6.685			9.37	4.51	6.94		
RP_2	S_2	4.45	8.66	6.555			4.62	8.99	6.805		
1.0g	S_3	12.3	7.14	9.72			12.77	7.42	10.095		
	S_4	3.31	2.4	2.855			3.44	2.49	2.965		
	S_5	0	2.3	1.15			0	2.38	1.19		
Rp_2*VAM		5.818	4.968		5.393		6.04	5.158		5.599	
	S_1	11.27	8.77	10.02			11.7	9.1	10.4		
Rp_3	S_2	6.51	19.66	13.085			6.76	20.42	13.59		
2.0g	S_3	12.51	12.8	12.655			12.99	13.29	13.14		
	S_4	2.27	5.36	3.815			2.36	5.56	3.96		
	S_5	0	5.2	2.6			0	5.4	2.7		
Rp_3*VAM		6.512	10.358		8.435		6.762	10.754		8.758	
	S_1	6.042	3.898			4.97	6.272	4.046			5.159
	S_2	3.164	8.702			5.933	3.286	9.036			6.161
S*VAM	S_3	7.732	4.672			6.202	8.028	4.852			6.44
	S_4	1.798	4.08			2.939	1.868	4.236			3.052
	S_5	2.49	2.25			2.37	2.586	2.336			2.461
VAM		7.0753	7.8673				7.3467	8.1687			
LSD at 0.05	RP= ------- S= 0.02563 VAM = 0.000315 RP*S*VAM= 3.452						RP=------ S= 0.0.0415 VAM = 0.00036 RP*S*VAM= 3.964				

2.5. Total dry weight (TDW) (g)

Seedlings treated with S_2 displayed the highest TDW in both seasons (7.991 and 8.344g for first and second season, respectively), while the seedlings which applied with S_5 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 11.568g), 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 Rp_3 and amended with S_3 in both seasons (26.335 and 28.649g for first and second season, respectively), (Table 7).

2.6. Shoot/ root ratio (SRR)

Application of RP the seedlings which treated with RP_3 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 RP_2 displayed the highest SRR (0.468) in the first season, yet in the second season the inoculated seedlings with mycorrhiza and treated with RP_3 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). Na Cl 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_3 + 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 (53.86and 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

		First season					Second season				
Rock phosphate RP	**Salinity level (ppm)**	**VAM inoculation**		**RP*S**	**RP**	**S**	**VAM inoculation**		**RP*S**	**RP**	**S**
		C	**VAM**				**C**	**VAM**			
	S_1	11.741	9.251	10.496			11.897	10.148	11.023		
Rp_1	S_2	6.886	18.873	12.880			6.829	19.298	13.264		
0.0g	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		10.080	11.017		10.548		10.278	11.963		11.120	
	S_1	12.421	9.117	10.769			12.632	8.752	10.692		
RP_2	S_2	8.122	11.076	9.599			8.152	11.135	9.644		
1.0g	S_3	13.885	9.155	11.520			14.295	9.209	11.752		
	S_4	1.202	3.049	2.126			1.156	3.045	2.101		
	S_5	0.000	3.957	1.979			0.000	3.852	1.926		
Rp_2*VAM		7.788	7.291		7.539		7.935	7.221		7.578	
	S_1	3.272	2.687	2.980			1.953	3.302	2.628		
Rp_3	S_2	14.542	11.457	13.000			13.653	12.402	13.028		
2.0g	S_3	14.385	26.335	16.756			15.629	28.649	17.555		
	S_4	4.509	9.065	6.787			3.959	10.114	7.037		
	S_5	0.000	6.467	3.234			0.000	6.958	3.479		
Rp_3*VAM		8.608	14.494		11.551		8.558	15.521		12.039	
	S_1	7.741	5.965			6.853	7.636	6.260			6.948
	S_2	4.884	11.098			7.991	4.872	11.816			8.344
S*VAM	S_3	8.596	6.906			7.751	9.012	7.143			8.077
	S_4	2.644	5.499			4.072	2.558	5.930			4.244
	S_5	2.573	3.171			2.872	2.659	3.355			3.007
VAM		8.825	10.934				8.924	11.568			
LSD at	RP: --------		S= 0.0.321		VAM = 1.457		RP= ------				S= 0.73254
0.05	RP*S*VAM= 4.3522						VAM = 1.814		RP*S*VAM= 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

		First season					Second season				
Rock phosphate RP	**Salinity level (ppm)**	**VAM inoculation**		**RP*S**	**RP**	**S**	**VAM inoculation**		**RP*S**	**RP**	**S**
		C	**VAM**				**C**	**VAM**			
	S_1	0.185	0.450	0.289			0.156	0.533	0.304		
Rp_1	S_2	0.417	0.242	0.285			0.352	0.287	0.303		
0.0g	S_3	0.062	0.827	0.213			0.053	0.979	0.236		
	S_4	0.232	0.209	0.214			0.196	0.247	0.237		
	S_5	0.033	0.448	0.129			0.028	0.529	0.144		
RP_1*VAM		0.133	0.331		0.229		0.113	0.392		0.247	
	S_1	0.376	1.101	0.611			0.348	0.941	0.541		
RP_2	S_2	0.825	0.279	0.464			0.765	0.239	0.417		
1.0g	S_3	0.129	0.282	0.185			0.119	0.241	0.164		
	S_4	0.363	0.312	0.342			0.336	0.267	0.307		
	S_5	0.000	0.720	0.720			0.000	0.618	0.618		
Rp_2*VAM		0.339	0.468		0.398		0.314	0.400		0.353	
	S_1	0.290	0.306	0.297			0.167	0.363	0.253		
Rp_3	S_2	0.290	0.306	0.297			0.167	0.363	0.253		
2.0g	S_3	0.150	0.494	0.324			0.203	0.466	0.336		
	S_4	0.986	0.691	0.779			0.678	0.819	0.777		
	S_5	0.000	0.244	0.244			0.000	0.289	0.289		
Rp_3*VAM		0.322	0.399		0.52		0.266	0.443		0.375	
	S_1	0.281	0.530			0.379	0.218	0.547			0.347
	S_2	0.543	0.275			0.347	0.483	0.308			0.354
S*VAM	S_3	0.112	0.478			0.250	0.123	0.472			0.254
	S_4	0.471	0.348			0.385	0.369	0.400			0.390
	S_5	0.033	0.409			0.212	0.028	0.436			0.222
VAM		0.247	0.390				0.215	0.416			
LSD at 0.05	RP: 0.00235 S= 0.00241 VAM = 0.06632 RF*VAM =0.05647 RP*S*VAM= 0.00233						RP= 0.00246 S= 0.00415 AM = 0.14114 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

		First season					Second season				
Rock phosphate RP	**Salinity level (ppm)**	**VAM inoculation**		**RP*S**	**RP**	**S**	**VAM inoculation**		**RP*S**	**RP**	**S**
		C	**VAM**				**C**	**VAM**			
	S1	70.36	78.2	74.28			74.58	84.46	79.52		
Rp_1	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_1*VAM		64.48	72.81		68.64		68.35	78.63		73.49	
	S1	68.34	76.54	72.44			72.44	82.66	77.55		
RP_2	S2	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		

Cont.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

		First season					Second season				
Rock phosphate RP	**Salinity level (ppm)**	**VAM inoculation**		**RP*S**	**RP**	**S**	**VAM inoculation**		**RP*S**	**RP**	**S**
		C	**VAM**				**C**	**VAM**			
Rp_2*VAM		65.09	71.89		68.49		69	77.64		73.32	
	S1	71.78	79.98	75.88			76.09	86.38	81.235		
Rp_3	S2	69.22	77.42	73.32			73.37	83.61	78.49		
2.0g	S3	68.72	76.92	72.82			72.84	83.07	77.955		
	S4	65.9	74.1	70			69.85	80.03	74.94		
	S5	0	71.51	35.76			0	77.23	38.615		
Rp_3*VAM		55.124	75.99		65.557		58.43	82.06		70.245	
	S1	70.16	78.24			74.2	74.37	84.5			79.44
	S2	67.07	74.94			71.01	71.1	80.94			76.02
S*VAM	S3	66.55	74.08			70.31	70.54	80			75.27
	S4	63.89	71.09			67.49	67.72	76.77			72.25
	S5	40.16	69.46			54.81	42.56	75.02			58.79
VAM		61.56	73.56				65.26	79.44			
LSD at	RP: ------	S= 3.026		VAM = 4.523			RP= ------	S= 3.0798		VAM = 4.555	
0.05	RP*S* = 1.521			RP*S*VAM= 4.0025			RP*S =1.5411		RP*S*VAM= 4.0.369		

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

		First season					Second season				
Rock phosphate RP	**Salinity level (ppm)**	**VAM inoculation**		**RP*S**	**RP**	**S**	**VAM inoculation**		**RP*S**	**RP**	**S**
		C	**VAM**				**C**	**VAM**			
	S1	33.02	110.36	71.69			33.02	34.67	33.85		
Rp1	S2	44.35	51.44	47.90			44.35	46.57	45.46		
0.0g	S3	36.45	43.36	39.91			36.45	38.27	37.36		
	S4	36.77	36.52	36.65			36.77	38.61	37.69		
	S5	21.54	35.41	28.48			21.54	22.62	22.08		
0.0*VAM		34.43	55.42		44.92		34.43	36.15		35.29	
	S1	34.92	107.36	71.14			34.92	36.67	35.80		
RP2	S2	44.65	49.56	47.11			44.65	46.88	45.77		
1.0g	S3	38.45	37.36	37.91			38.45	40.37	39.41		
	S4	39.77	31.66	35.72			39.77	41.76	40.77		
	S5	24.54	26.21	25.38			24.54	25.77	25.16		
Rp2*VAM		36.47	50.43		43.45		36.47	38.29		37.38	
	S1	78.92	146.97	112.95			76.04	177.33	126.69		
Rp3	S2	48.65	54.06	51.36			46.13	59.33	52.73		
2.0g	S3	48.45	37.00	42.73			46.78	42.04	44.41		
	S4	39.77	22.11	30.94			35.66	28.60	32.13		
	S5	0.00	18.56	9.28			0.00	20.45	10.23		
Rp3*VAM		43.16	55.74		49.45		40.92	65.55		53.24	
	S1	48.95	121.56			85.26	47.99	82.89			65.44
	S2	45.88	51.69			48.79	45.04	50.93			47.99
S*VAM	S3	41.12	39.24			40.18	40.56	40.23			40.39
	S4	38.77	30.10			34.43	37.40	36.32			36.86
	S5	15.36	26.73			21.04	15.36	22.95			19.15
VAM		38.02	53.86				37.27	46.66			
LSD at	RP: ------	S= 2.088		VAM = 4.654			RP= ------	S= 3.0798		VAM = 4.847	
0.05	RP*S* = 1.521			RP*S*VAM= 4.0025			RP*S =1.5411		RP*S*VAM= 4.0.369		

4.2. Proline content (g/10u0g).

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 Na Cl (S_5) 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 oliefera* under salinity condition in both seasons (Table 13), while nitrogen increased significantly only with the third level of salinity S_3, Phosphorous concentration increased was significantly under the fourth level of salinity S_4 (Tables 11 and 12). Regardless, the effect of salinity and RP application, the inoculated seedlings with VAM fungus displayed the highest values in N (2.95 and 3.07% for first and second season, respectively), P (0.52 and 0.50% for first and second season, respectively) and K content (%) (1.97 and 1.85% for first and second season, respectively, (Tables, 11, 12 and 13). Furthermore, the significant interaction between RP application and symbiosis agent has manifested the highest values of 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 (0.70and 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

		First season					Second season				
Rock phosphate RP	**Salinity level (ppm)**	**VAM inoculation**		**RP*S**	**RP**	**S**	**VAM inoculation**		**RP*S**	**RP**	**S**
		C	**VAM**				**C**	**VAM**			
	S1	1.31	5.38	3.35			1.28	5.50	3.39		
Rp_1	S2	3.42	7.28	5.35			3.35	7.45	5.40		
0.0g	S3	3.43	9.47	6.45			3.36	9.85	6.61		
	S4	4.45	9.74	7.10			4.32	10.13	7.23		
	S5	12.37	18.96	15.67			12.00	19.72	15.86		
RP_1*VAM		5.00	10.17		7.58		4.86	10.53		7.70	
	S1	1.61	6.45	4.03			1.56	8.00	4.78		
RP_2	S2	4.62	9.68	7.15			4.48	12.00	8.24		
1.0g	S3	5.17	10.89	8.03			4.60	13.50	9.05		
	S4	5.45	10.74	8.10			4.85	13.32	9.09		
	S5	0.00	18.96	9.48			0.00	23.51	11.76		
Rp_2*VAM		3.37	11.34		7.36		3.10	14.07		8.58	
	S1	1.11	6.77	3.94			1.23	8.26	4.75		
Rp_3	S2	3.02	9.78	6.40			3.35	13.69	8.52		
2.0g	S3	3.63	11.63	7.63			4.03	16.28	10.16		
	S4	3.77	11.89	7.83			4.18	16.65	10.42		
	S5	10.88	20.04	15.46			12.08	28.06	20.07		
Rp_3*VAM		4.48	12.02		8.25		4.97	16.59		10.78	
	S1	1.34	6.20			3.77	1.36	7.25			4.31
	S2	3.69	8.91			6.30	3.73	11.05			7.39
S*VAM	S3	4.08	10.66			7.37	4.00	13.21			8.60
	S4	4.56	10.79			7.67	4.45	13.37			8.91
	S5	7.75	19.32			13.54	8.03	23.76			15.90
VAM		4.28	11.18				4.31	13.73			
LSD at	RP: 1.0365					S= 3.652	RP=1.521				S= 4.877
0.05	VAM = 5.654		RP*S*VAM= 5..369				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

		First season					Second season				
Rock phosphate RP	**Salinity level (ppm)**	**VAM inoculation**		**RP*S**	**RP**	**S**	**VAM inoculation**		**RP*S**	**RP**	**S**
		C	**VAM**				**C**	**VAM**			
	S1	1.92	2.10	2.01			1.80	1.97	1.89		
Rp_1	S2	2.21	2.66	2.44			2.08	2.50	2.29		
0.0g	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		
RP_1*VAM		1.91	2.53		2.22		1.79	2.37		2.08	
	S1	1.22	2.93	2.08			1.26	2.99	2.13		
RP_2	S2	1.66	2.78	2.22			1.71	2.83	2.27		
1.0g	S3	1.66	2.96	2.31			1.71	3.02	2.37		
	S4	1.33	2.98	2.16			1.37	3.04	2.21		
	S5	1.22	2.85	2.04			1.26	2.91	2.09		
Rp_2*VAM		1.42	2.90		2.16		1.46	2.96		2.21	
	S1	1.88	2.21	2.05			2.54	2.98	2.76		
Rp_3	S2	1.63	3.41	2.52			2.20	3.98	3.09		
2.0g	S3	2.21	4.34	3.28			2.98	4.19	3.59		
	S4	3.25	4.02	3.64			4.39	3.98	4.19		
	S5	0.00	3.10	1.55			0.00	4.19	2.10		
Rp_3*VAM		1.79	3.42		2.61		2.42	3.86		3.14	
	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
S*VAM	S3	2.03	3.20			2.62	2.26	3.13			2.69
	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		1.71	2.95				1.89	3.07			
LSD at	RP= -------		S= 0.0942		VAM = 1.0214		RP= -------		S=0.0632	VAM = 1.0547	
0.05	RP*S*VAM= 0.3478						RP*S*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

		First season					Second season				
Rock phosphate RP	**Salinity level (ppm)**	**VAM inoculation**		**RP*S**	**RP**	**S**	**VAM inoculation**		**RP*S**	**RP**	**S**
		C	**VAM**				**C**	**VAM**			
	S_1	0.25	0.39	0.32			0.25	0.37	0.31		
Rp_1	S_2	0.19	0.46	0.33			0.22	0.44	0.33		
0.0g	S_3	0.19	0.45	0.32			0.19	0.43	0.31		
	S_4	0.29	0.54	0.42			0.28	0.52	0.40		
	S_5	0.29	0.36	0.33			0.28	0.35	0.32		
RP_1*VAM		0.24	0.44		0.34		0.24	0.42		0.33	
	S_1	0.27	0.39	0.33			0.26	0.37	0.32		
RP_2	S_2	0.20	0.52	0.36			0.20	0.50	0.35		
1.0g	S_3	0.17	0.45	0.31			0.17	0.43	0.30		
	S_4	0.19	0.38	0.29			0.19	0.36	0.28		
	S_5	0.24	0.34	0.29			0.24	0.33	0.29		

Cont. Table 13.

		First season					Second season				
Rock phosphate RP	**Salinity level (ppm)**	**VAM inoculation**		**RP*S**	**RP**	**S**	**VAM inoculation**		**RP*S**	**RP**	**S**
		C	**VAM**				**C**	**VAM**			
Rp_2*VAM		0.21	0.42		0.32		0.21	0.40		0.31	
	S_1	0.27	0.38	0.33			0.26	0.36	0.31		
Rp_3	S_2	0.59	0.62	0.61			0.58	0.65	0.62		
2.0g	S_3	0.19	0.83	0.51			0.19	0.84	0.52		
	S_4	0.41	1.46	0.94			0.40	1.40	0.90		
	S_5	0.00	0.20	0.10			0.00	0.19	0.19		
Rp_3*VAM		0.29	0.70		0.50		0.29	0.69		0.51	
		0.26	0.39			0.33	0.26	0.37			0.31
		0.33	0.53			0.43	0.33	0.53			0.43
S*VAM		0.18	0.58			0.38	0.18	0.57			0.38
		0.30	0.79			0.55	0.29	0.76			0.53
		0.18	0.30			0.24	0.17	0.29			0.26
VAM		0.25	0.52				0.25	0.50			
LSD at	RP= -------					S= 0.1963	RP= -------		S= 0.1063	VAM = 0.187	
0.05	VAM = 0.198			RP*S*VAM= 0.2155			RP*S*VAM= 0.2474				

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 S_4 (1.46 and 1.40% for first and second season, respectively), respectively. (Table 12).

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 RP_3(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 absorpable 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 Alikamanoglu (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 increaed chlorophyll content due to VAM inoculation under normal as well as salinity stress corroborates the reports of Aroca *et al.* (2013) in lettuce, Alqarawi *et al.* (2014) in *Tamarixy aphylla* and Abd_Allah *et al.* (2015) in *Sesbania sesban*. Recently, in salt- stressed *Brassica juncea*, Ahmad *et al.* (2015)

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).

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

		First season					Second season				
Rock phosphate RP	**Salinity level (ppm)**	**VAM inoculation**		**RP*S**	**RP**	**S**	**VAM inoculation**		**RP*S**	**RP**	**S**
		C	**VAM**				**C**	**VAM**			
	S_1	1.96	1.99	1.98			1.82	1.87	1.85		
Rp_1	S_2	1.84	1.87	1.86			1.71	1.76	1.74		
0.0g	S_3	1.65	1.86	1.76			1.53	1.75	1.64		
	S_4	1.86	1.75	1.81			1.73	1.65	1.69		
	S_5	1.62	1.71	1.67			1.51	1.61	1.56		
RP_1*VAM		1.79	1.84		1.81		1.66	1.73		1.69	
	S_1	2.04	2.10	2.07			1.90	1.97	1.94		
RP_2	S_2	1.86	1.87	1.87			1.73	1.76	1.75		
1.0g	S_3	1.81	1.90	1.86			1.68	1.79	1.74		
	S_4	1.93	1.93	1.93			1.79	1.81	1.80		
	S_5	1.63	1.68	1.66			1.52	1.58	1.55		
Rp_2*VAM		1.85	1.90		1.88		1.72	1.78		1.75	
	S_1	1.84	2.22	2.03			1.71	2.09	1.90		
Rp_3	S_2	1.81	2.34	2.08			1.68	2.20	1.94		
2.0g	S_3	1.81	2.51	2.16			1.68	2.36	2.02		
	S_4	1.82	1.95	1.89			1.69	1.83	1.76		
	S_5	0.00	1.82	0.91			0.00	1.71	0.86		
Rp_3*VAM	S_1	1.46	2.17		1.81		1.35	2.04		1.70	
	S_2	1.95	2.10			2.03	1.81	1.98			1.89
	S_3	1.84	2.03			1.93	1.71	1.91			1.81
S*VAM	S_4	1.76	2.09			1.92	1.63	1.97			1.80
	S_5	1.87	1.88			1.87	1.74	1.76			1.75
		1.08	1.74			1.41	1.01	1.63			1.32
VAM		1.70	1.97				1.58	1.85			
LSD at 0.05	RP= ------- S= 0.527 VAM = 0.152 RP*S*VAM= 0.3148						RP= ------- S= 0.694 VAM = 0.163 RP*S*VAM= 0.3784				

Table 15. Sodium content Na (%) of inoculated and uninoculated seedlings of *Moringa oleifera* Lam. with VAM, unfertilized and fertilized with RP under five levels of salinity

		First season					Second season				
Rock phosphate RP	**Salinity level (ppm)**	**VAM inoculation**		**RP*S**	**RP**	**S**	**VAM inoculation**		**RP*S**	**RP**	**S**
		C	**VAM**				**C**	**VAM**			
	S_1	0.49	0.42	0.46			0.49	0.39	0.44		
Rp_1	S_2	0.49	0.46	0.48			0.49	0.43	0.46		
0.0g	S_3	0.56	0.55	0.56			0.57	0.51	0.54		
	S_4	0.62	0.58	0.60			0.63	0.54	0.59		
	S_5	0.73	0.66	0.70			0.74	0.61	0.68		
RP_1*VAM		0.58	0.53		0.56		0.58	0.50		0.54	
	S_1	0.49	0.42	0.46			0.49	0.39	0.44		
RP_2	S_2	0.49	0.46	0.48			0.49	0.43	0.46		
1.0g	S_3	0.56	0.49	0.53			0.57	0.46	0.52		
	S_4	0.57	0.54	0.56			0.58	0.50	0.54		
	S_5	0.70	0.60	0.65			0.71	0.56	0.64		
Rp_2*VAM		0.56	0.50		0.53		0.57	0.47		0.52	
	S_1	0.44	0.42	0.43			0.44	0.39	0.42		
Rp_3	S_2	0.49	0.46	0.48			0.49	0.43	0.46		
2.0g	S_3	0.53	0.48	0.51			0.54	0.45	0.50		
	S_4	0.63	0.55	0.59			0.64	0.51	0.58		
	S_5	0.00	0.60	0.30			0.00	0.56	0.28		
Rp_3*VAM	S_1	0.42	0.50		0.46		0.42	0.47		0.45	
	S_2	0.47	0.42			0.45	0.47	0.39			0.43
	S_3	0.49	0.46			0.48	0.49	0.43			0.46
S*VAM	S_4	0.55	0.51			0.53	0.56	0.47			0.52
	S_5	0.61	0.56			0.58	0.62	0.52			0.57
		0.48	0.62			0.55	0.48	0.58			0.53
VAM		0.52	0.51				0.52	0.48			
LSD at	RP: 0.02		S=0.094		VAM = 0.0076		RP= 0.03				S= 0.075
0.05	RP*S*VAM= 0.13						VAM = 0.0095		RP*S*VAM= 0.16		

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 (Elevin *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 RP_2 (1g/kg soil) to enhance its growth and to gain tolerance against salinity stress.

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الملخص العربي

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

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الملوحة عامل إجهاد بيئى خطير يؤثر بشدة على نمو النباتات وتكشفها, وتسبب ملوحة التربة غالبا تأخير الإنتاجية النباتية فى كلا الحالتين الطبيعية والزراعية. فطر الميكوريزا الحويصلية الشجيرية المتكافلة يمكن أن يقلل من الإجهاد النباتى الناتج عن الملوحة وذلك عن طريق زيادة درجة التحمل للملوحة.

أجري هذا البحث فى موسمي نمو بدءاً من يونيه ٢٠١٧ حتى مايو ٢٠١٨ وتم تكرار التجربة فى نفس المواعيد فى الموسم التالي وذلك بمشتل قسم الغابات وتكنولوجيا الأخشاب بمحطة البحوث الزراعية – كلية الزراعة – جامعة الإسكندرية بمنطقة ابيس وكذلك معامل قسم الغابات وتكنولوجيا الأخشاب بالكلية.

أوضحت النتائج المتحصل عليها أن التلقيح بواسطة VAM وإضافة صخر الفوسفات أدت إلى تشجيع جوهرى للنمو معبراً عنها فى الحيوية وارتفاع النبات والوزن الجاف للمجموع الخضرى والوزن الجاف للجذر ونسبة الساق الى الجذر والوزن الجاف الكلى والعناصر المعدنية فى الأوراق N, P, K وانخفاض فى تركيز الصوديوم فى الأوراق مقارنه بالشتلات غير الملقحة بالميكوريزا.

أثرت معاملات كلوريد الصوديوم على محتوى الأوراق من الكلوروفيل حيث انخفض تركيز كلوروفيل أ وب فى الأوراق فى كلا موسمى النمو.

اوضحت النتائج أن أعلى تركيز للعناصر المعدنية فى الاوراق كانت فى حالة المعاملة الثنائية للشتلات بالميكوريزا وإضافة صخر الفوسفات.

كما اظهرت النتائج أن أعلى تركيز للبرولين فى شتلات المورينجا الملحقة بالميكوريزا فى كلا موسمى النمو .كما اتضح من التجربة أن شتلات المورينجا يمكنها أن تتحمل تركيز ملحى عالى يصل إلى ١٧١ ميلليمول فى وجود الميكوريزا.

ينصح بأن يتم تلقيح شتلات المورينجا بفطر الميكوريزا وإضافة صخر الفوسفات بمعدل (١جم / كجم تربة). وذلك لزيادة سرعة النمو واكساب الشتلات تحملا للإجهاد الملحى.

الكلمات الدالة: ملوحة– برولين– ميكوريزا– مورينجا– صخر الفوسفات.