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Improving Tolerance of *Vachellia farnesiana* Plants to Irrigation Water Salinity by Using Bio-Inocula under Sandy Soil Conditions

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Abstract: This study was conducted at the Experimental Laboratory of the Natural Resources Department, Inst. of African Res. and Studies, Cairo Univ., Giza, Egypt and Experimental Nursery of Ornamental Horticulture Department, Faculty of Agric., Cairo Univ., Giza, Egypt during the two successive seasons of 2013/2014 and 2014/2015. The aim of this study was to investigate the possibility of improving tolerance of *Vachellia farnesiana* plants grown in sandy soil to irrigation water salinity by using bio-inocula. Seedlings of *Vachellia farnesiana* were inoculated with mixed spores of arbuscular mycorrhizal fungi (AM) from genera *Glomus*, *Gigaspora* and *Acaulospora* (450 spores/g sterilized peatmoss as a carrier) at the rate of 10g /pot alone, with two different *Sinorhizobium terangae* strains of bacteria (R, 10^9 CFU/ml) at the rate of 10 ml/ pot, or with mixed spores of arbuscular mycorrhizal fungi (AM) followed by the two *Sinorhizobium terangae* strains after the mycorrhizal symbiosis was established (AM+ R). The plants were irrigated (till 85% of field capacity) every 4 days using tap water (control, 300 ppm salinity) or tap water containing a mixture of NaCl and $CaCl_2$ (1:1 w/w) at concentrations of 3000, 6000, 9000 or 12000 ppm. Increasing irrigation water salinity concentration significantly reduced survival percentage, plant height, stem diameter, number of branches/plant, root length as well as fresh and dry weights of branches + leaves and roots/plant, total chlorophylls, carotenoids, N, P and K% in leaves comparing with the control. Raising the salt concentration in irrigation water up to 6000 ppm resulted in an insignificant reduction in most of the studied characters as compared to the control, whereas higher salt concentrations of 9000 and 12000 ppm recorded significant reduction. Total carbohydrates, Na, Cl, Ca, proline and activity of ascorbate peroxidase, catalase, superoxide dismutase and peroxidase in leaves were increased by raising the salt concentration in irrigation water salinity compared to the control. Plants receiving AM+R inocula gave the significantly highest mean values for most of the studied characteristics followed by plants receiving AM inoculum, then that inoculated with R inoculum, whereas uninoculated plants (control) gave the lowest values. On contrary uninoculated plants had the highest values of Na, Ca and Cl% in their leaves followed by that inoculated with R, AM and AM+R inocula. It can be concluded that AM+R treatment was the best inoculum tested for improving tolerance of *Vachellia farnesiana* plants grown in sandy soil to irrigation water salinity up to 6000 ppm without significant reduction than the plants irrigated with tap water. The anatomical study proved this conclusion because of showing an obvious increase in number of stomata on the upper surface of leaf blade and prominent increments in thickness of leaf blade, palisade tissue, spongy tissue as well as midrib over than those of control.

Key words: *Vachellia farnesiana* · Arbuscular mycorrhizal fungi · Biofertilizer · *Sinorhizobium* · Salinity · Irrigation · NaCl · $CaCl_2$ · Stomata · Leaf blade thickness · Anatomical characters · Microscopical counts and measurements

INTRODUCTION

In Egypt, agriculture is an important contributor to economic growth. It consumes more than 85% of the total demand for water. The major problems facing reclamation of new lands are the shortage of freshwater resources, increasing groundwater salts which lead to increasing salinity of irrigation water, deficient in soil constituents

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and lack of macro and micronutrients [1, 2]. Salination is a critical problem nowadays. 97.5 percent of the water worldwide is too salty for human consumption and plant production [3]. So, some strategies are needed in these areas to overcome salinity stress on plants like growing salt tolerant plants, inoculation with arbuscular mycorrhizal fungi and/or *Sinorhizobium terangae* nitrogen-fixing bacteria.

The deleterious effect of salinity on the plant is attributed to the presence of high concentrations of soluble salts in root zone soil moisture, causing a negative impact on plant morphology and growth, as well as yield and chemical composition. Water salinity damages plants due to salt toxicity and dehydration caused by low water potential. Soluble salts have high osmotic pressures which restrict the uptake of water by the roots and cause a reduction in root growth and elongation; thereby inhibit hormonal signals generated by the roots which accordingly reduce plant growth [4]. Salinity induces the synthesis of abscisic acid and closing of stomata when transported to guard cells decreases leading to a decline in photosynthesis. Also, abscisic acid inhibits cell expansion. Salinity decreases the respiration rate and fatty acid content of the plant. Excessive sodium ions at the root surface inhibit potassium uptake by the root. Potassium deficiency retards root growth because potassium plays a critical role in maintaining cell turgor, membrane potential and enzyme activities. Accumulation of high Na^+ in the cytosol causes high Na^+: K^+ ratio, which result in obstructing enzymatic functions activated by K^+ in cells [5] as well as stomatal opening and closing because when potassium moves into the guard cells, water flows and the cells swell, then the pores open and allow gaseous exchange [6]. Phosphorus and magnesium content significantly decreased as salinity increased [7]. Na^+ and Cl^- cause ion toxicity of the plant; competes with nutrients such as K^+, Ca^{2+}, NO_3^- and decrease N, P, K and Mg levels in medicinal plants as *Foeniculum vulgare* and *Achillea fragratissima* [8, 9]. Meanwhile, salinity increases the amino acid proline [10]. Salinity induces anatomical and cytological changes in plant organs. The anatomical modifications are related to metabolic adaptations to survive in the presence of salinity. In leaves, salinity results in smaller epidermal cells; smaller stomata and reduction of stomatal area and leaf area to reduce the transpiration surface to save water [11]. Munns [4] described the events in a plant exposed to salinity. Cells lose water and shrink, cell elongation rates are reduced, changes in cell elongation and cell division lead to smaller formed leaves and the plant does not form lateral shoots and may die. Salinity stress causes oxidation, resulting in producing reactive oxygen species (ROS) which interact with cellular components, causing damage to lipids in the cell membrane and other cellular structures. The increased activities of the antioxidant enzymes superoxide dismutase, catalase, ascorbate peroxidase, non-specific peroxidase and glutathione reductase are related to the resistance to salinity stress. Enzymatic antioxidant molecules alleviate cellular damage caused by ROS [12-14]. Growing salt-tolerant and moderately salt tolerant plants are one of the solutions for improving plant growth and production exposed to salts in water irrigation. Salt tolerant plants accumulate compatible solutes in their cells and have a low rate of Na^+ and Cl^- transport to the leaves [4].

Vachellia farnesiana (L.) Willd, commonly known as needle bush, is a deciduous tree belonging to the family *Fabaceae*. It is a fast growing tree that grows all over the world. It has flexible ecological requirements, it is suitable for sandy, loamy and clay soils and can grow in nutritionally poor soil. Its suitable pH is acid, neutral and alkaline soils. The trees have also been used for erosion control in sandy soils [15]. It has multipurpose uses, the bark and pods are used as astringent and demulcent in medicine for its tannin content and used in making dyes and inks; the leaves and roots are used for medicinal purposes. The foliage is used as fodder. Flowers of the plant give through distillation the perfume essence (Cassie) which is extensively used in European perfumery. Seeds are pressed to make oil for cooking. The plant is used for fencing posts, agricultural implements, pegs, woodenware [16].

Some agricultural practices such as inoculation with arbuscular mycorrhizal fungi (AMF) as a natural biofertilizer are obligate symbionts with the host plant. They increase soil aggregate stability by using their thick extraradical hyphal network and secret glycoproteins (glomalin and glomalin related proteins) to compact the soil particles [17]. AMF increase the plant uptake of immobile phosphate ions from the soil as well as N, K, Mg and some micronutrients leading to stimulating growth [18, 19]. It regulates the synthesis and distribution of plant hormones [20]. Arbuscular mycorrhizae are bio-ameliorators of saline soils by reducing the oxidative stress in plants resulted from ion toxicity and osmotic imbalances caused by salinity. They accelerate the leaching of NaCl, decrease the percentage of exchangeable sodium and the electrical conductivity and increase both water filtration and holding capacity. Also, arbuscular mycorrhizae increase tolerance to salinity stress. They help plants to adapt to salinity stress by regulating abscisic acid as well as inducing the

antioxidant catalase, total peroxidase and phosphatase enzymes activity in response to salinity stress [21-23]. They increase root and shoot biomass, phosphorus and nitrogen contents, K^+: Na^+, Ca^{2+}: Na^+ ratios, accumulation of proline and glycine betaine and antioxidant enzyme activities in stressed plants. It reduces lipid peroxidation [24].

Sinorhizobium terangae is a nitrogen-fixing bacterium. Zou *et al*. [25] found that *Acacia ampliceps* plant inoculated with salt-tolerant *Rhizobium* PMA63/1 under 200 mM NaCl was less affected concerning growth, nodule number and N content per plant than plants inoculated with PMA37. Inoculation with a salt-tolerant *Rhizobium* strain may improve biological N_2-fixation under saline conditions.

Therefore, the aim of this study was to assess the effect of inoculation with arbuscular mycorrhizal fungi and/or *Sinorhizobium terangae* bacteria on vegetative growth and anatomical characteristics as well as chemical constituents and enzyme activities of *Vachellia farnesiana* plant grown in sandy soil under irrigation water salinity.

MATERIALS AND METHODS

This study was conducted at the Experimental Laboratory of the Natural Resources Department, Inst. of African Res. and Studies, Cairo Univ., Giza, Egypt and Experimental Nursery of Ornamental Horticulture Department, Faculty of Agric., Cairo Univ., Giza, Egypt during the two seasons of 2013/2014 and 2014/2015. The aim of the study was to investigate the role of arbuscular mycorrhizal fungi and *Sinorhizobium terangae* inoculation in improving tolerance of *Vachellia farnesiana* plants to irrigation water salinity.

Seeds from the crushed pods of *Vachellia farnesiana* were pre-treated with He-Ne laser at irradiance 1.70 W/cm^2 for 9 min to accelerate germination [26], then the seeds were sown on 1st June 2013 and 2014 (in the first and second seasons, respectively), in 25-cm plastic pots filled with aerated steam sterilized sandy soil obtained from Alwadi Alfaregh, Sixth of October desert, Giza, Egypt. The physical and chemical characteristics of the soil are shown in Table 1. The layout of the experiment was a split-plot design, with the main plots arranged in a randomized complete blocks design, with 3 blocks (replicates). The main plots were assigned to the inoculation treatments, while the sub-plots were assigned to the irrigation water salinity treatments. The study included 20 treatments [4 inoculation treatments X 5 salt concentrations, including the control], with each block consisting of 100 plants (5 plants/treatment). After one month of seed sowing, the first quarter of the total number of seedlings was inoculated with mixed spores of arbuscular mycorrhizal (AM) fungi from genera *Glomus*, *Gigaspora* and *Acaulospora* (450 spores/g sterilized peatmoss as a carrier) at the rate of 10g /pot alone [27]. The second quarter was inoculated with a bacterial suspension of two different strains of *Sinorhizobium terangae* bacteria (R, 10^9 CFU/ml) at the rate of 10 ml/ pot. The third quarter was inoculated with mixed spores of arbuscular mycorrhizal fungi (AM) as described above. After the mycorrhizal symbiosis was established, the two different *Sinorhizobium terangae* strains (R) were applied. The fourth quarter was left without inoculation as a control. The above two bio-inocula were obtained from Soil, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt. The pots were irrigated with tap water from the seed sowing till one month after inoculation to allow the time required for the symbiosis to occur. On 1st August, the irrigation with saline water was initiated. The plants were irrigated every 4 days using tap water as a control (300 ppm salinity) or saline water at concentrations of 3000, 6000, 9000 or 12000 ppm until the termination of each season. The different saline water concentrations were prepared using a mixture of NaCl and $CaCl_2$ (1:1, w/w). Plants were irrigated till 85% of soil field capacity (F.C.) in each irrigation. The soil moisture tension was measured before each irrigation using microtensiometer and the quantity of water needed to reach 85% F.C. was calculated as described by Richards [28].

On 1st May, 2014 and 2015 (in the two seasons, respectively), the experiment was terminated and the survival percentage was recorded. Also, vegetative characters including plant height, stem diameter (at 5 cm above soil surface), the number of branches /plant, main root length, as well as fresh and dry weights of branches + leaves and roots/plant were recorded. Samples of fresh leaves were taken at random from each treatment. Chemical analysis of fresh leaf samples was conducted to determine their total chlorophylls and carotenoids content using the method described by Moran [29]. The proline content in fresh leaves was proceeded according to Bates *et al*. [30]. The enzymes of ascorbate peroxidase, catalase, peroxidase and superoxide dismutase were extracted and their activities were assayed. Ascorbate peroxidase activity was measured according to Yamaguchi *et al*. [31] and catalase activity was assayed by measuring the initial rate of hydrogen peroxide disappearance according to Velikova *et al*. [32]. Superoxide dismutase activity was assayed by monitoring

Table 1: Physical and chemical characteristics of the soil used for growing *Vachellia farnesiana* (average of both seasons)

Physical analysis												
Clay %			Silt %			Fine sand%			Coarse sand%			Texture class
2.9			1.1			39.6			56.4			Sandy
Chemical analysis												
			Soluble cations and anions (meq/L)							Available elements (ppm)		
pH	EC dS/m	O.M%	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+	N	P	K
7.8	1.1	0.34	1.6	5.2	1.5	3.7	2.1	2.3	0.2	10	6	200

the inhibition of the photochemical reduction of nitroblue tetrazolium, based on the method of Yu and Rengel [33]. Peroxidase activity was based upon the method as described by Srinivas *et al*. [34]. The content of total carbohydrates in the oven dried (70°C) leaf samples was determined using the method described by Dubois *et al.* [35]. Also, dried leaf samples were digested to extract nutrients as described by Piper [36] and the extract was analyzed to determine N% using the modified micro-Kjeldahl method as described by Pregl [37], phosphorus % [38], K and Na % were assayed using the flame spectrophotometer [39], Ca % was determined by atomic absorption [40] and Cl % was determined according to Higinbothan *et al*. [41].

For microscopic counts and measurements, investigated materials included the leaf blade of the upper fifth leaf on the main stem. Specimens were taken during the second growing season from plants, eleven months age, treated by 6000 ppm of salinity only, AM+R inoculum only and AM+R inoculum under 6000 ppm of salinity, in addition to the control plants. These treatments showed remarkable morphological and chemical responses. An impression of upper leaf blade epidermis was prepared according to the replica technique (conservative facsimile technique) as described by Prakash *et al.* [42]. Three slides were microscopically examined and the mean number of stomata per microscopic field area (unit area) at 500x magnification was accounted on the upper leaf blade surface. Microtechnique procedures were carried out according to Sass [43]. Specimens were kept for killing and fixation in formalin acetic acid ethyl alcohol solution (F.A.A.). The fixed specimens were then washed in 50% ethyl alcohol, dehydrated in normal butyl alcohol series and finally embedded in paraffin wax of 56-58°C. Specimens of twenty microns thickness were cut using a rotary microtome. Sections were stained with safranin fast green combination and then cleared in xylene before mounting in Canada balsam. Thereafter, three prepared slides were subjected to microscopic analysis at 50x magnification and the mean values of measurements were calculated.

The data obtained for the vegetative characteristics and chemical constituents were subjected to statistical analysis of variance and the means were compared using the Least Significant Difference (L.S.D.) test at the 5% level, as described by Little and Hills [44]. The survival percentage data was arcsine transformed and the transformed data was statistically analyzed.

RESULTS AND DISCUSSION

Survival Percentage: The results presented in Table (2) showed the effect of irrigation water salinity and bio-inocula treatments on the survival percentage of *Vachellia farnesiana* plants. In both seasons, in most cases, increasing irrigation water salinity concentration resulted in a steady reduction in the mean survival percentage. However, this reduction was insignificant when plants irrigated with saline water up to 6000 ppm in both seasons. In the first season, the salt concentration of 3000 ppm in the irrigation water statistically caused no reduction in the survival percentage, compared to the control. Salt concentrations of 9000 and 12000 ppm significantly reduced the recorded values, compared to the control. A similar reduction in the survival percentage as a result of salt stress has been reported by prior studies [45-47].

Data presented in Table 2 showed that in both seasons, *Vachellia farnesiana* plants receiving the mixed spores of arbuscular mycorrhizal fungi and two different *Sinorhizobium terangae* strains (AM+R) treatment significantly gave the highest mean value for survival percentage than control followed by plants that inoculated with mixed spores of arbuscular mycorrhizal (AM) fungi inoculums then that received two different *Sinorhizobium terangae* strains (R) with no significant difference among them. Generally, uninoculated plants (control) gave the lowest survival percentage. The results obtained are in agreement with prior study on *Acacia farnesiana* [48].

Table 2: Effect of irrigation water salinity and bio- inocula on survival percentage, plant height (cm), stem diameter (mm), number of branches/plant and main root length of *Vachellia farnesiana* during 2013/2014 and 2014/2015 seasons

	1st season					2nd season				
	Inoculum (I)					Inoculum (I)				
Salt conc. (SC) ppm	Control	AM	R	AM+R	Mean (SC)	Control	AM	R	AM+R	Mean (SC)
					*Survival percentage					
Control	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a	100 a
3000 ppm	100 a	100 a	100 a	100 a	100 a	90.0 ab	90.0 ab	90.0 ab	100 a	92.5 a
6000 ppm	93.3 ab	93.3 ab	93.3ab	100 a	95.0 a	80.0 a-c	90.0 ab	90.0 ab	93.3 ab	89.6 a
9000 ppm	73.3 de	86.7 bc	80.0 cd	90.0 a-c	82.5 b	70.0 bc	80.0 a-c	80.0 a-c	80.0 a-c	77.5 b
12000 ppm	60.0 f	66.7 e f	66.7 e f	80.0 cd	68.4 c	60.0 c	70.0 bc	60.0 c	80.0 a-c	67.5 b
Mean (I)	85.3 b	89.3 ab	88.0 ab	94.0 a	----	80.0 b	87.0 ab	84.0 ab	90.7a	----
					Plant height (cm)					
Control	57.1	89.3	82.2	98.1	81.7	70.4	94.8	89.7	112.3	91.8
3000 ppm	54.6	86.9	81.8	95.8	79.8	63.0	92.6	89.5	109.1	88.6
6000 ppm	50.1	82.8	78.3	92.4	75.9	57.8	92.3	85.9	105.1	85.3
9000 ppm	44.9	81.9	73.4	91.7	73.0	54.9	90.9	81.4	104.7	83.0
12000 ppm	40.9	80.4	72.9	89.1	70.8	51.3	87.9	80.3	102.0	80.4
Mean (I)	49.5	84.3	77.7	93.4	----	59.5	91.7	85.4	106.6	----
LSD (0.05)										
SC			6.5					7.5		
I			5.1					6.5		
SC X I			11.9					14.2		
					Stem diameter (mm)					
Control	7.8	11.0	9.5	13.3	10.4	8.9	14.1	12.7	15.3	12.8
3000 ppm	7.2	10.8	9.1	13.0	10.0	8.2	13.7	12.2	15.0	12.3
6000 ppm	6.5	10.3	8.6	12.1	9.4	7.3	11.2	11.5	14.5	11.1
9000 ppm	6.0	10.1	8.2	11.1	8.9	6.5	10.5	10.5	13.2	10.2
12000 ppm	5.5	9.7	7.8	10.5	8.4	6.0	10.4	10.0	11.8	9.6
Mean (I)	6.6	10.4	8.6	12.1	----	7.4	12.0	11.4	14.0	----
LSD (0.05)										
SC			1.1					1.5		
I			1.0					1.1		
SC X I			2.1					2.5		
					Number of branches /plant					
Control	3.6	5.4	5.1	6.5	5.2	4.4	6.6	5.9	7.6	6.1
3000 ppm	2.4	4.6	3.5	5.0	3.9	2.8	4.8	4.5	6.7	4.7
6000 ppm	2.0	3.3	3.1	4.5	3.2	1.7	3.8	3.8	5.5	3.7
9000 ppm	1.5	3.0	2.7	3.7	2.7	1.1	3.0	2.7	4.4	2.8
12000 ppm	1.3	2.4	2.0	3.1	2.2	1.0	2.5	2.3	3.5	2.3
Mean (I)	2.2	3.7	3.3	4.6	----	2.2	4.1	3.8	5.5	----
LSD (0.05)										
SC			1.4					1.5		
I			1.0					1.2		
SC X I			2.4					2.9		
					Main root length (cm)					
Control	32.5	51.5	45.0	62.2	47.8	35.0	56.8	52.7	68.7	53.3
3000 ppm	32.1	51.3	44.7	62.0	47.5	34.5	56.5	52.2	68.5	52.9
6000 ppm	28.5	49.5	42.0	60.5	45.1	31.0	54.8	49.3	67.2	50.6
9000 ppm	24.5	49.1	40.7	58.5	43.2	30.5	53.7	46.8	65.8	49.2
12000 ppm	23.0	47.0	38.2	58.2	41.6	26.3	50.3	40.5	65.3	45.6
Mean (I)	28.1	49.7	42.1	60.3	----	31.5	54.4	48.3	67.1	----
LSD (0.05)										
SC			3.3					3.8		
I			3.1					3.4		
SC X I			6.7					7.8		

AM: mixed spores of arbuscular mycorrhizal fungi from genera *Glomus*, *Gigaspora* and *Acaulospora*

R: Two different *Sinorhizobium terangae* strains

Table 3: Effect of irrigation water salinity and bio- inocula on fresh and dry weights of branches+ leaves and roots/plant of *Vachellia farnesiana* during 2013/2014 and 2014/2015 seasons

	1st season					2nd season				
	Inoculum (I)					Inoculum (I)				
Salt conc. (SC) ppm	Control	AM	R	AM+R	Mean (SC)	Control	AM	R	AM+R	Mean (SC)
	Fresh weight of branches + leaves (g/plant)									
Control	141.5	226.7	208.2	263.7	210.0	139.2	232.0	206.6	270.1	212.0
3000 ppm	141.0	224.5	196.6	242.9	201.3	135.1	202.4	196.1	262.7	199.1
6000 ppm	120.8	217.8	191.2	229.8	189.9	112.4	199.2	178.9	256.2	186.7
9000 ppm	114.8	209.5	183.3	223.2	182.7	97.5	180.5	166.8	226.7	167.9
12000 ppm	96.6	187.0	175.0	210.0	167.2	75.7	176.4	153.0	219.4	156.1
Mean (I)	122.9	213.1	190.9	233.9	----	112.0	198.1	180.3	247.0	----
LSD (0.05)										
SC			23.7					25.9		
I			20.5					21.4		
SCX I			44.1					47.3		
	Dry weight of branches + leaves (g/plant)									
Control	26.17	46.93	43.30	56.17	43.14	32.27	51.57	48.23	60.87	48.24
3000 ppm	25.37	46.47	42.67	55.90	42.60	31.53	51.27	47.77	60.67	47.81
6000 ppm	20.17	43.13	38.43	54.30	39.01	25.90	47.40	42.97	58.07	43.59
9000 ppm	16.87	42.11	34.52	51.57	36.27	19.82	44.81	38.44	56.11	39.80
12000 ppm	14.00	39.27	33.30	51.37	34.49	18.80	42.93	37.33	55.03	38.52
Mean (I)	20.52	43.58	38.44	53.86	----	25.66	47.60	42.95	58.15	----
LSD (0.05)										
SC			5.30					4.82		
I			4.61					4.10		
SCX I			10.61					10.33		
	Fresh weight of roots (g/plant)									
Control	65.6	146.6	137.3	198.0	136.9	72.3	131.6	100.5	158.7	115.8
3000 ppm	63.9	143.7	129.6	182.4	129.9	72.2	120.1	87.5	146.3	106.5
6000 ppm	45.8	124.6	125.3	176.9	118.2	65.9	96.9	74.2	137.9	93.7
9000 ppm	34.6	115.4	89.5	142.9	95.6	38.2	72.8	65.2	81.8	64.5
12000 ppm	32.6	84.3	75.8	102.8	73.9	36.2	61.9	54.0	70.8	55.7
Mean (I)	48.5	122.9	111.5	160.6	----	57.0	96.7	76.3	119.1	----
LSD (0.05)										
SC			10.1					11.4		
I			8.2					10.1		
SCX I			21.8					23.7		
	Dry weight of roots (g/plant)									
Control	15.96	32.85	28.15	40.40	29.34	16.77	27.11	22.51	34.11	25.13
3000 ppm	13.70	29.74	24.75	38.01	26.55	15.01	25.05	20.73	30.41	22.80
6000 ppm	10.49	27.17	23.06	35.84	24.14	13.11	19.47	15.87	28.87	19.33
9000 ppm	8.09	23.88	20.13	33.45	21.39	8.11	14.20	13.52	16.77	13.15
12000 ppm	7.00	23.17	18.65	20.97	17.45	8.09	12.94	12.55	15.64	12.31
Mean (I)	11.05	27.36	22.95	33.73	----	12.22	19.75	17.04	25.16	----
LSD (0.05)										
SC			3.11					4.47		
I			2.01					2.11		
SCX I			6.71					6.88		

AM: mixed spores of arbuscular mycorrhizal fungi from genera *Glomus*, *Gigaspora* and *Acaulospora*

R: Two different *Sinorhizobium terangae* strains.

Regarding interaction between the effects of irrigation water salinity and bio-inocula treatments, data recorded in the two seasons (Table 2) showed that no significant difference was recorded between the values of the survival percentage of both uninoculated plants and that received AM+R, AM or R bio-inocula and irrigated with saline water with concentration up to 6000 ppm. In the first season, increasing irrigation water salinity to 9000 or 12000 ppm resulted in significant reduction in survival percentage compared to the highest value

recorded (100%) except for the plants inoculated with AM+R and irrigated with 9000 ppm saline water which resulted in an insignificant reduction in survival percentage compared to the highest value recorded. In the second season, increasing irrigation water salinity to 9000 ppm resulted in significant reduction in survival percentage of uninoculated plants compared to the highest value recorded (100%), whereas both uninoculated plants and that received AM or R bio-inocula and irrigated with saline water at a concentration of 12000 ppm resulted in significant reduction in survival percentage compared to the highest value recorded (100%).

Vegetative Characteristics

Effect of Irrigation Water Salinity: Data presented in Tables 2 and 3 revealed that increasing the salt concentration in the irrigation water had an adverse effect on the studied vegetative characteristics including plant height, stem diameter, the number of branches/plant, main root length as well as fresh and dry weights of branches + leaves and roots/plant. In both seasons, the mean values recorded for the studied vegetative characteristics of *Vachellia farnesiana* plants were reduced steadily as the used concentration was raised (3000, 6000, 9000 or 12000 ppm). However, 6000 ppm caused a reduction in the mean values recorded for plant height, root length as well as fresh and dry weights of branches + leaves/plant in both seasons as well as stem diameter in the first season as compared to the control. Higher salt concentrations (9000 or12000 ppm) resulted in significant reduction in the values recorded for all studied characteristics as compared to the control.

Raising the salt concentration in irrigation water salinity up to 3000 ppm resulted in insignificant reduction in vegetative characteristics including stem length, stem diameter in the second season in addition to the number of branches/plant as well as fresh and dry weights of roots/plant in both seasons compared to the control, higher salt concentrations (6000-12000 ppm) resulted in significant reduction in the values recorded for these characteristics as compared to the control. The results obtained are in agreement with previous studies [49-51]. Generally, results revealed that salt concentration treatments up to 6000 ppm had an insignificant effect on most of the vegetative growth parameters.

Effect of Bio-Inocula: Data presented in Tables 2 and 3 showed that, in both seasons, *Vachellia farnesiana* plants receiving the mixed spores of arbuscular mycorrhizal fungi and two different *Sinorhizobium terangae* strains (AM+R) treatment gave the highest mean values for all vegetative parameters followed by plants receiving mixed spores of arbuscular mycorrhizal (AM) fungi inoculum, then that inoculated with two different *Sinorhizobium terangae* strains (R) with significant differences among them in most cases. Uninoculated plants (control) gave the significantly lower values for all studied characteristics compared with other treatments. Similar results were recorded by other investigations [52- 58].

No significant difference was recorded between values of plant height, stem diameter, fresh weight of branches + leaves/plant in the second season as well as, number of branches/plant in both seasons for plants received mixed spores of arbuscular mycorrhizal (AM) fungi inoculums or that inoculated with different strains (R) treatments. Also, in the first season, there was no significant difference between the values of the number of branches/plant for plants received AM+R treatment and that received AM treatment.

Interaction Between Effects of Irrigation Water Salinity and Bio-inocula Treatments: The data recorded in the two seasons (Tables 2 and 3) showed that the most vigorous vegetative characters of *Vachellia farnesiana* plants (i.e., the highest values for the different vegetative characteristics) were obtained as a result of inoculation with the mixed spores of arbuscular mycorrhizal fungi followed by two different *Sinorhizobium terangae* strains (AM+R) and irrigation with tap water. In most cases, within each irrigation water salinity treatment, inoculation with the mixed spores of arbuscular mycorrhizal fungi followed by two different *Sinorhizobium terangae* strains (AM+R) resulted in the highest values of the vegetative characteristics, followed by inoculation with the mixed spores of arbuscular mycorrhizal fungi (AM), then inoculation with the different *Sinorhizobium terangae* strains (R), whereas control (uninoculated) plants gave the lowest values. Within each inoculation treatment, increasing irrigation water salinity resulted in a steady reduction in the values of vegetative parameters.

Plants receiving the mixed spores of arbuscular mycorrhizal fungi + the two different *Sinorhizobium terangae* strains (AM+R) and irrigated with saline water at 6000 ppm gave insignificantly lower values of stem diameter, number of branches/plant and fresh and dry weights of roots/plant than the highest values recorded with the plants inoculated with the same inoculum and irrigated with tap water, whereas plants received the same

inocula and irrigated with saline water at 9000 ppm gave insignificantly lower values of fresh weight of branches + leaves/plant than the highest values recorded with the plants inoculated with the same inoculum and irrigated with tap water. Moreover, plants received the same inocula and irrigated with saline water at 12000 ppm gave insignificantly lower values of plant height, root length and dry weight of branches+leaves/plant than the highest values recorded with the plants inoculated with the same inoculum and irrigated with tap water.

From the above results, it can be concluded that although the most vigorous characters of *Vachellia farnesiana* plants can be obtained with inoculation with the mixed spores of arbuscular mycorrhizal fungi and the two different *Sinorhizobium terangae* strains used (AM+R) and irrigation using tap water. It is clear that inoculation with the same inoculum (AM+R) increased the tolerance to irrigation water salinity up to 6000 ppm with no significant reduction in all studied vegetative characteristics compared to the highest values recorded with the plants inoculated with the same inoculum and irrigated with tap water. Results are in harmony with previous works [59-63].

Chemical Composition

Leaf Pigment Contents [Total Chlorophylls (A+b) and Carotenoids]: Data presented in Table (4) revealed that increasing the salt concentration in the irrigation water had an adverse effect on the total chlorophylls and carotenoids contents in the leaves of *Vachellia farnesiana* plants as compared with control. In both seasons, total chlorophylls and carotenoids contents were reduced steadily as the salt concentration was raised to 3000, 6000, 9000 or 12000 ppm. However, raising the salt concentration in irrigation water up to 6000 ppm resulted in an insignificant reduction in the mean values recorded for total chlorophylls and carotenoids contents as compared to the control, whereas higher salt concentrations (9000 or12000 ppm) resulted in significant reduction in the values recorded as compared to the control. Similar reductions in the chlorophylls content as a result of irrigation using saline water were stated by previous studies [45, 51, 64-66].

Data presented in Table (4) revealed that in both seasons, total chlorophylls (a+b) and carotenoids contents were clearly affected by bio- inocula, as compared to the control plants. *Vachellia farnesiana* plants receiving the mixed spores of arbuscular mycorrhizal fungi followed by two different *Sinorhizobium terangae* strains (AM+R) gave the significantly higher mean values for total chlorophylls and carotenoids contents in both seasons followed by plants receiving mixed spores of arbuscular mycorrhizal (AM) fungi inocula, then that inoculated with two different *Sinorhizobium terangae* strains (R) with significant differences among them. Uninoculated plants (control) gave the significantly lowest contents of leaf pigments investigated compared with other treatments. Similar results were reported by previous studies [53, 62, 67, 68].

Data recorded in the two seasons (Table 4) showed that the highest contents of total chlorophylls and carotenoids in leaves of *Vachellia farnesiana* plants were obtained as a result of inoculation with the mixed spores of arbuscular mycorrhizal fungi and two *Sinorhizobium terangae* strains (AM+R) and irrigation with tap water. On the other hand, uninoculated plants irrigated with saline water at the highest concentration (12000 ppm) had the lowest chlorophylls and carotenoids contents in the leaves compared with other treatments. Within each irrigation water salinity treatment, inoculation with the mixed spores of arbuscular mycorrhizal fungi followed by two *Sinorhizobium terangae* strains (AM+R) resulted in the highest values of the total chlorophylls and carotenoids content, followed by inoculation with the the mixed spores of arbuscular mycorrhizal fungi (AM), then inoculation with the two *Sinorhizobium terangae* strains (R), whereas control (uninoculated) plants gave the lowest values. In most cases, within each inoculation treatment, increasing irrigation water salinity resulted in steady reductions in the total chlorophylls and carotenoids contents.

It is worth mentioning that, in both seasons, plants receiving the (AM+R) bio- inoculum and irrigated with saline water up to 6000 ppm gave insignificantly lower values for total chlorophylls and carotenoids contents as compared to the highest values recorded with the plants inoculated with the same inoculum and irrigated with tap water.

Total Carbohydrates (% Dry Weight of Leaves): Data shown in Table (4) revealed that, in both seasons, the lowest total carbohydrates percentage was recorded in leaves of *Vachellia farnesiana* plants irrigated with tap water (control). Increasing salt concentration in the irrigation water steadily increased the total carbohydrates percentage. The highest total carbohydrates percentage was recorded by using irrigation water salinity at 12000 ppm. The obtained results are in agreement with prior findings [62, 69, 70].

Table 4: Effect of irrigation water salinity and bio- inocula on total chlorophylls, carotenoids (mg/g fresh weight) and total carbohydrates (% dry weight) in leaves of *Vachellia farnesiana* during 2013/2014 and 2014/2015 seasons

	1st season					2nd season				
	Inoculum (I)					Inoculum (I)				
Salt conc. (SC) ppm	Control	AM	R	AM+R	Mean (SC)	Control	AM	R	AM+R	Mean (SC)
	Total chlorophylls (mg/g fresh weight)									
Control	1.37	2.36	1.89	2.70	2.08	1.49	2.57	2.16	2.83	2.26
3000 ppm	1.33	2.34	1.87	2.69	2.06	1.44	2.55	2.13	2.81	2.23
6000 ppm	1.08	2.19	1.68	2.61	1.89	1.16	2.36	1.91	2.69	2.03
9000 ppm	1.02	1.98	1.75	2.30	1.76	1.11	1.66	1.45	1.98	1.55
12000 ppm	0.76	2.00	1.45	2.15	1.59	0.84	1.40	1.34	1.77	1.34
Mean (I)	1.11	2.17	1.73	2.49	----	1.21	2.11	1.80	2.42	----
LSD (0.05)										
SC			0.26					0.26		
I			0.21					0.23		
SCX I			0.51					0.54		
	Carotenoids (mg/g fresh weight)									
Control	0.63	1.15	0.91	1.31	1.00	0.71	1.23	1.03	1.38	1.09
3000 ppm	0.63	1.13	0.89	1.30	0.99	0.67	1.21	1.00	1.36	1.06
6000 ppm	0.50	1.06	0.82	1.27	0.91	0.53	1.15	0.90	1.30	0.97
9000 ppm	0.43	0.97	0.71	1.07	0.80	0.44	1.12	0.79	1.15	0.88
12000 ppm	0.33	0.91	0.68	1.05	0.74	0.38	1.06	0.77	1.11	0.83
Mean (I)	0.50	1.04	0.80	1.20	----	0.55	1.15	0.90	1.26	----
LSD (0.05)										
SC			0.13					0.15		
I			0.11					0.11		
SCX I			0.23					0.26		
	Total carbohydrates (% dry weight)									
Control	14.67	28.50	19.33	31.67	23.54	16.43	25.33	24.51	31.50	24.44
3000 ppm	14.33	29.00	20.00	32.00	23.83	17.57	26.07	25.33	32.72	25.42
6000 ppm	19.67	31.00	25.67	34.00	27.59	25.67	35.33	32.67	36.08	32.44
9000 ppm	20.11	32.55	26.07	35.42	28.54	26.22	36.98	35.34	38.01	34.14
12000 ppm	24.67	35.00	29.33	36.00	31.25	29.33	37.67	35.67	38.67	35.33
Mean (I)	18.69	31.21	24.08	33.82	----	23.04	32.28	30.70	35.40	----
LSD (0.05)										
SC			2.46					2.98		
I			2.34					2.54		
SCX I			4.97					5.86		

AM: mixed spores of arbuscular mycorrhizal fungi from genera *Glomus*, *Gigaspora* and *Acaulospora*

R: Two different *Sinorhizobium terangae* strains

The mean total carbohydrates percentage in leaves of *Vachellia farnesiana* plants was affected by the bio- inocula (Table 4) in both seasons as compared to the control plants. In both seasons, *Vachellia farnesiana* plants receiving the mixed spores of arbuscular mycorrhizal fungi and two different *Sinorhizobium terangae* strains (AM+R) gave the significantly highest mean values for total carbohydrates percentages followed by plants receiving the mixed spores of arbuscular mycorrhizal fungi (AM) inoculum, then that inoculated with two *Sinorhizobium terangae* strains (R) with significant differences among them, in most cases. Uninoculated plants (control) gave the significantly lowest contents of total carbohydrates. The obtained results are in agreement with prior findings [58, 70, 71]

Regarding the effect of different combinations of irrigation water salinity and bio-inoculation treatments, it is clear from the results presented in Table (4) that the highest total carbohydrates percentages were obtained in both seasons when the plants received the mixed spores of arbuscular mycorrhizal fungi followed by two *Sinorhizobium terangae* strains (AM+R) and irrigated with saline water at the concentration of 12000 ppm.

On the other hand, the lowest values were recorded in both seasons with uninoculated plants irrigated with tap water. Within each irrigation water salinity treatment, inoculation with the mixed spores of arbuscular mycorrhizal fungi followed by two *Sinorhizobium terangae* strains (AM+R) resulted in the highest values of carbohydrates percentage in leaves, followed by inoculation with the mixed spores of arbuscular mycorrhizal fungi (AM), then inoculation with the two *Sinorhizobium terangae* strains (R), whereas control (uninoculated) plants gave the lowest values. In most cases, within each inoculation treatment, increasing irrigation water salinity resulted in a steady increase in the total carbohydrates percentages.

In both seasons, plants receiving the mixed spores of arbuscular mycorrhizal fungi followed by two different *Sinorhizobium terangae* strains (AM+R) and irrigated with saline water at a concentration of 6000 ppm resulted in insignificantly lower values than the highest values recorded with plants received the same inoculum and irrigated with saline water at the concentration of 12000 ppm.

c- N, P and K (% Dry Weight of Leaves): The results in Table (5) showed that regardless the effect of inoculation treatments, the N, P and K (% dry weight of leaves) were decreased steadily with raising the salt concentration. Accordingly, the lowest percentages of the three nutrients were found in plants irrigated with saline water at a concentration of 12000 ppm compared with control. However, increasing the salt concentration in irrigation water up to 6000 ppm resulted in insignificant reduction in N, P and K% as compared to the control, in most cases. The only exception to this trend was recorded in the first season with plants irrigated with saline water at 6000 ppm which resulted in significantly lower P% as compared to the control. Generally, higher salt concentrations (9000 and 12000 ppm) resulted in significant reduction in the percentages recorded as compared to the control. These results are in agreement with previous studies [45, 62, 69, 72].

The results presented in Table (5) showed that in both seasons, *Vachellia farnesiana* plants receiving AM+R inocula had the significantly highest N, P and K percentages in their leaf tissues, followed by plants receiving AM and R inocula, respectively while uninoculated plants had the lowest percentages of the three main nutrients. In the first season, there was no significant difference between plants received AM+R or AM inocula for their N% in leaves. These results are in agreement with prior findings [73, 74].

Data presented in Table (5) also revealed that in most cases, within each irrigation water salinity treatment, plants inoculated with the AM+R inoculum had the highest N, P and K%, followed by plants inoculated with AM and R inocula respectively, then the uninoculated plants. Also, in most cases, within each inoculation treatment, increasing irrigation water salinity resulted in a steady decrease in N, P and K% . In both seasons, the highest N, P and K% were recorded in leaves of plants that received the AM+R inoculum and were irrigated with tap water, whereas uninoculated plants irrigated with saline water at the concentration of 12000 ppm had the lowest percentages of the three elements. It is worth mentioning that, in both seasons, plants receiving the AM+R bio- inoculum and irrigated with saline water at 6000 ppm gave insignificantly lower percentages for the three elements as compared to the highest values recorded with the plants inoculated with the same inoculum and irrigated with tap water.

It is worth mentioning that treating the plants with AM+R inoculum under 6000 ppm irrigation water salinity resuled in significant higher percentages for N, P and K compared with plants treated with 6000 ppm salinity only. In this regard, the ability of plants to counteract stress depends on the level of K^+ in the plant, plants with high level of potassium are salt tolerants [75, 76].

d- Na, Ca and Cl (% Dry Weight of Leaves): The results in Table (6) showed that Na, Ca and Cl (% of the dry weight of leaves) were increased steadily with raising the salt concentration in both seasons. Accordingly, the lowest percentages of the three elements were found in plants irrigated with tap water, whereas the highest percentages were found in plants irrigated with saline water at the concentration of 12000 ppm, regardless of the effect of inoculation treatments. However, increasing the salt concentration in irrigation water up to 3000 ppm resulted in an insignificant increase in the mean values recorded for Na and Cl% as compared to the control, whereas the same irrigation water salinity level resulted in a significant increase in the mean values recorded for Ca% as compared to the control. Generally, higher salt concentrations (6000-12000 ppm) resulted in a significant increase in the values recorded as compared to the control. These results are in agreement with results obtained in other studies [51, 77-78].

Table 5: Effect of irrigation water salinity and bio- inocula on N, P and K (% dry weight) in leaves of *Vachellia farnesiana* during 2013/2014 and 2014/2015 seasons

	1st season					2nd season				
	Inoculum (I)					Inoculum (I)				
Salt conc. (SC) ppm	Control	AM	R	AM+R	Mean (SC)	Control	AM	R	AM+R	Mean (SC)
					N (% dry weight)					
Control	1.48	1.71	1.59	1.84	1.66	1.76	2.01	1.89	2.22	1.97
3000 ppm	1.35	1.67	1.54	1.74	1.58	1.65	1.88	1.77	2.12	1.86
6000 ppm	1.31	1.64	1.50	1.69	1.54	1.60	1.83	1.70	2.07	1.80
9000 ppm	1.29	1.59	1.40	1.60	1.47	1.43	1.62	1.53	1.68	1.57
12000 ppm	1.22	1.49	1.32	1.55	1.40	1.33	1.54	1.47	1.60	1.49
Mean (I)	1.33	1.62	1.47	1.69	----	1.55	1.78	1.67	1.94	----
LSD (0.05)										
SC			0.15					0.18		
I			0.11					0.12		
SCX I			0.27					0.30		
					P (% dry weight)					
Control	0.19	0.27	0.21	0.31	0.25	0.18	0.25	0.20	0.34	0.24
3000 ppm	0.18	0.24	0.20	0.29	0.23	0.17	0.23	0.20	0.31	0.23
6000 ppm	0.15	0.21	0.18	0.24	0.20	0.16	0.19	0.19	0.27	0.20
9000 ppm	0.14	0.16	0.15	0.19	0.16	0.16	0.18	0.18	0.22	0.19
12000 ppm	0.12	0.15	0.14	0.16	0.14	0.14	0.17	0.15	0.20	0.17
Mean (I)	0.16	0.21	0.18	0.24	---	0.16	0.20	0.18	0.27	----
LSD (0.05)										
SC			0.05					0.05		
I			0.02					0.03		
SCX I			0.08					0.08		
					K (% dry weight)					
Control	1.40	1.54	1.46	1.60	1.50	1.42	1.66	1.56	1.82	1.62
3000 ppm	1.33	1.44	1.34	1.53	1.41	1.37	1.53	1.48	1.79	1.54
6000 ppm	1.21	1.34	1.32	1.51	1.35	1.26	1.49	1.39	1.68	1.46
9000 ppm	1.15	1.22	1.20	1.34	1.23	1.21	1.43	1.26	1.45	1.34
12000 ppm	1.10	1.18	1.16	1.23	1.17	1.11	1.31	1.24	1.40	1.27
Mean (I)	1.24	1.34	1.30	1.44	----	1.27	1.48	1.39	1.63	----
LSD (0.05)										
SC			0.16					0.17		
I			0.10					0.11		
SCX I			0.25					0.28		

AM: mixed spores of arbuscular mycorrhizal fungi from genera *Glomus*, *Gigaspora* and *Acaulospora*

R: Two different *Sinorhizobium terangae* strains.

The results presented in Table (6) showed that in both seasons, regardless the effect of salinity, uninoculated *Vachellia farnesiana* plants (control) had the highest Na, Ca and Cl% in the leaves than the other inoculation treatments followed by plants inoculated with two different *Sinorhizobium terangae* strains (R), mixed spores of arbuscular mycorrhizal (AM) fungi , meanwhile plants receiving the mixed spores of arbuscular mycorrhizal fungi and two different *Sinorhizobium terangae* strains (AM+R) inoculums gave the significantly lower values as compared to the control. There was no significant difference between plants inoculated with AM+R and AM in both seasons for Na% and Ca%, as well as Cl% in the second season. Also, there was no significant difference between plants inoculated with R inoculum and control in the first season for Ca% and in the second season for Cl%. Moreover, there was no significant difference among plants received AM or R inoculum for Cl% in the first season.

Data presented in Table (6) showed that, in most cases, within each irrigation water salinity treatment the lowest values of Na, Ca and Cl% were recorded with plants received AM+R inoculum followed by AM and R respectively, compared with uninoculated plants which

Table 6: Effect of irrigation water salinity and bio- inocula on Na, Ca and Cl (% dry weight) as well as proline (µmoles/g fresh weight) in leaves of *Vachellia farnesiana* during 2013/2014 and 2014/2015 seasons

	1st season					2nd season				
	Inoculum (I)					Inoculum (I)				
Salt conc. (SC) ppm	Control	AM	R	AM+R	Mean (SC)	Control	AM	R	AM+R	Mean (SC)
					Na (% dry weight)					
Control	0.35	0.36	0.33	0.34	0.35	0.33	0.32	0.31	0.31	0.32
3000 ppm	0.52	0.40	0.46	0.38	0.44	0.51	0.37	0.39	0.34	0.40
6000 ppm	0.75	0.55	0.62	0.50	0.61	0.63	0.46	0.52	0.42	0.51
9000 ppm	0.82	0.61	0.72	0.60	0.69	0.90	0.70	0.82	0.62	0.76
12000 ppm	0.96	0.74	0.81	0.70	0.80	0.96	0.82	0.87	0.78	0.86
Mean (I)	0.68	0.53	0.59	0.50	----	0.67	0.53	0.58	0.49	----
LSD (0.05)										
SC			0.11					0.12		
I			0.08					0.06		
SCX I			0.19					0.18		
					Ca (% dry weight)					
Control	0.46	0.45	0.48	0.46	0.46	0.37	0.38	0.36	0.39	0.38
3000 ppm	0.65	0.59	0.61	0.50	0.59	0.61	0.50	0.57	0.42	0.53
6000 ppm	0.71	0.63	0.68	0.56	0.65	0.65	0.53	0.57	0.52	0.57
9000 ppm	0.76	0.69	0.74	0.66	0.71	0.79	0.62	0.71	0.56	0.67
12000 ppm	0.85	0.76	0.81	0.71	0.78	0.87	0.67	0.76	0.62	0.73
Mean (I)	0.69	0.62	0.66	0.58	----	0.66	0.54	0.60	0.50	----
LSD (0.05)										
SC			0.11					0.09		
I			0.07					0.06		
SCX I			0.18					0.15		
					Cl (% dry weight)					
Control	0.21	0.24	0.25	0.23	0.23	0.35	0.34	0.37	0.37	0.36
3000 ppm	0.35	0.30	0.33	0.26	0.31	0.49	0.39	0.46	0.38	0.43
6000 ppm	0.49	0.37	0.41	0.29	0.39	0.59	0.49	0.53	0.45	0.52
9000 ppm	0.62	0.41	0.49	0.37	0.47	0.78	0.67	0.72	0.62	0.70
12000 ppm	0.79	0.59	0.62	0.42	0.61	0.85	0.72	0.81	0.66	0.76
Mean (I)	0.49	0.38	0.42	0.31	----	0.61	0.52	0.58	0.50	----
LSD (0.05)										
SC			0.09					0.11		
I			0.05					0.05		
SCX I			0.14					0.17		
					Proline (µ moles/g fresh weight)					
Control	2.3	2.6	2.5	2.2	2.4	2.7	3.7	3.1	3.5	3.3
3000 ppm	3.7	6.2	4.8	9.8	6.1	3.8	6.9	5.7	14.8	7.8
6000 ppm	6.2	9.5	7.9	13.8	9.4	6.8	9.2	8.4	19.8	11.1
9000 ppm	10.2	15.8	14.2	18.9	14.8	9.1	16.8	14.9	23.8	16.2
12000 ppm	13.5	17.9	16.2	23.8	17.9	11.8	20.8	18.7	32.7	21.0
Mean (I)	7.2	10.4	9.1	13.7	----	6.8	11.5	10.2	18.9	----
LSD (0.05)										
SC			1.8					1.7		
I			1.1					1.3		
SCX I			2.9					3.2		

AM: mixed spores of arbuscular mycorrhizal fungi from genera *Glomus*, *Gigaspora* and *Acaulospora*

R: Two different *Sinorhizobium terangae* strains

resulted in the highest values in both seasons. Within each inoculation treatment in both seasons, increasing irrigation water salinity resulted in a steady increase in Na, Ca and Cl% in the leaves. So, in both seasons, the highest Na, Ca and Cl% in the leaves were recorded in leaves of uninoculated plants irrigated with saline water at the concentration of 12000 ppm. The results obtained are in agreement with other studies which found that *Glomus* genus of arbuscular mycorrhizal fungi can selectively take up potassium ions while avoids sodium uptake [79] and that inoculation with rhizobacteria under salt stress decreased sodium content in the plant [80].

Proline Content: Data presented in Table (6) showed that, in both seasons, *Vachellia farnesiana* plants irrigated with tap water had the significantly lowest proline contents, compared to other irrigation water salinity treatments. Increasing salt concentration in the irrigation water resulted in a steady increase in the mean proline content, regardless of the bio-inocula treatments. Accordingly, plants receiving the highest salt concentration (12000 ppm) gave the significantly highest mean values. Similar results were reported by prior studies [49, 72, 81].

Data presented in Table (6) revealed that, in both seasons, plants received the mixed spores of arbuscular mycorrhizal fungi and two different *Sinorhizobium terangae* strains (AM+R) inoculum gave the significantly highest values of proline content followed by plants received mixed spores of arbuscular mycorrhizal (AM) fungi inoculum, two different *Sinorhizobium terangae* strains (R) inoculum and then un-inoculated plants with significant differences among them. These results are in agreement with the previous study [24].

Regarding the effect of different combinations of irrigation water salinity and bio- inocula treatments, it is clear that, in both seasons, within each irrigation water salinity treatment, plants received the mixed spores of arbuscular mycorrhizal fungi and two different *Sinorhizobium terangae* strains (AM+R) inoculum gave the highest proline content followed by that received mixed spores of arbuscular mycorrhizal (AM) fungi, then that inoculated with two different *Sinorhizobium terangae* strains (R), whereas uninoculated plants had the lowest values in most cases. In both seasons, within each inoculation treatment, increasing irrigation water salinity resulted in a steady increase in the proline content. In both seasons, the significantly highest proline content was found in plants received AM+R inoculum and irrigated with 12000 ppm irrigation water salinity. On the other hand, plants received AM+R inoculum and irrigated with tap water had the lowest proline content in the first season, whereas uninoculated plants irrigated with tap water gave the lowest proline content in the second season.

Ascorbate Peroxidase, Catalase, Superoxide Dismutase and Peroxidase Activity: Data presented in Table (7) revealed that the activity of ascorbate peroxidase, catalase, superoxide dismutase and peroxidase (U mg^{-1} FW leaves) was increased steadily with raising the salt concentration. Accordingly, the lowest values were found in plants irrigated with tap water, whereas the highest activity was found in plants irrigated with saline water at the concentration of 12000 ppm, regardless of the effect of inoculation treatments.

The results presented in Table (7) showed that in both seasons, *Vachellia farnesiana* plants receiving the mixed spores of arbuscular mycorrhizal fungi and two different *Sinorhizobium terangae* strains (AM+R) inoculum had the highest activity of ascorbate peroxidase, catalase, superoxide dismutase and peroxidase (U mg^{-1} FW leaves) in the leaf tissues, followed by plants receiving mixed spores of arbuscular mycorrhizal (AM) fungi and two different *Sinorhizobium terangae* strains (R) inocula, while uninoculated plants had the lowest activity values comparing with other inoculation treatments. In this respect, previous studies found that mycorrhizal inoculation increased antioxidant enzymes in *Sesbania cannabina* and *Acacia nilotica* plants compared to nonmycorrhizal ones [64, 82].

Data presented in Table (7) showed that within each irrigation treatment the highest activity of ascorbate peroxidase, catalase, superoxide dismutase and peroxidase (U mg^{-1} FW leaves) in the leaf tissues was recorded with plants received AM+R inoculum followed by AM and R inocula, then uninoculated plants (control). Within each inoculation treatment, increasing irrigation water salinity resulted in a steady increase in activity of ascorbate peroxidase, catalase, superoxide dismutase and peroxidase (U mg^{-1} FW leaves) in the leaf tissues, in most cases. The highest values were recorded with plants inoculated with AM+R and irrigated with saline water at the concentration of 12000 ppm, whereas the lowest values were recorded with uninoculated plants irrigated with tap water.

Table 7: Effect of irrigation water salinity and bio- inocula on activity of ascorbate peroxidase, catalase, superoxide dismutase and peroxidasein (U mg^{-1} FW leaves) of *Vachellia farnesiana* during 2013/2014 and 2014/2015 seasons

	1st season					2nd season				
	Inoculum (I)					Inoculum (I)				
Salt conc. (SC) ppm	Control	AM	R	AM+R	Mean (SC)	Control	AM	R	AM+R	Mean (SC)
	Ascorbate peroxidase (U mg^{-1} FW leaves)									
Control	0.20	0.42	0.32	0.43	0.34	0.23	0.32	0.29	0.48	0.33
3000 ppm	0.23	0.47	0.36	0.51	0.39	0.25	0.39	0.32	0.51	0.37
6000 ppm	0.32	0.65	0.49	0.73	0.55	0.28	0.46	0.42	0.63	0.45
9000 ppm	0.48	0.87	0.68	0.89	0.73	0.34	0.52	0.49	0.71	0.52
12000 ppm	0.51	0.92	0.70	0.97	0.78	0.39	0.62	0.53	0.83	0.59
Mean (I)	0.35	0.67	0.51	0.71	----	0.30	0.46	0.41	0.63	----
	Catalase (U mg^{-1} FW leaves)									
Control	0.71	1.29	1.20	2.38	1.40	0.64	1.75	1.11	2.81	1.58
3000 ppm	0.98	3.45	2.11	3.68	2.56	1.12	1.86	1.58	2.98	1.89
6000 ppm	1.59	3.74	2.38	4.84	3.14	1.40	2.65	2.24	3.17	2.37
9000 ppm	2.45	3.95	2.74	4.25	3.35	2.32	4.34	3.42	4.71	3.70
12000 ppm	2.94	5.11	3.44	6.46	4.49	2.65	4.67	3.62	5.28	4.06
Mean (I)	1.73	3.51	2.37	4.32	----	1.63	3.05	2.39	3.79	----
	Superoxide dismutase (U mg^{-1} FW leaves)									
Control	0.51	2.41	1.73	4.21	2.22	0.48	2.46	1.21	4.08	2.06
3000 ppm	0.59	2.56	1.83	4.39	2.34	0.53	2.68	1.46	4.34	2.25
6000 ppm	0.74	2.75	2.24	4.68	2.60	0.68	2.81	1.89	4.62	2.50
9000 ppm	0.91	2.97	2.37	4.89	2.79	0.76	3.04	2.05	4.78	2.66
12000 ppm	1.11	3.21	2.39	4.98	2.92	0.94	3.44	2.85	4.86	3.02
Mean (I)	0.77	2.78	2.11	4.63	----	0.68	2.89	1.89	4.54	----
	Peroxidase (U mg^{-1} FW leaves)									
Control	0.82	1.45	1.21	2.11	1.30	0.71	1.42	1.12	1.65	1.23
3000 ppm	1.11	1.68	1.34	2.24	1.59	0.95	1.98	1.68	2.01	1.66
6000 ppm	1.37	2.85	2.24	2.98	2.36	1.21	2.24	1.98	3.98	2.35
9000 ppm	1.49	3.45	3.24	4.24	3.11	1.36	3.54	2.22	4.97	3.02
12000 ppm	2.11	4.84	3.70	5.88	4.13	1.68	3.93	2.65	5.24	3.38
Mean (I)	1.38	2.85	2.35	3.49	----	1.18	2.62	1.93	3.57	----

AM: mixed spores of arbuscular mycorrhizal fungi from genera *Glomus*, *Gigaspora* and *Acaulospora*
R: Two different *Sinorhizobium terangae* strains.

Microscopical Counts and Measurements: The present anatomical study is restricted to the control as well as the treatments of 6000 ppm salinity level, AM+R inoculum and AM+R inoculum under 6000 ppm of salinity which showed remarkable effects on plant morphology and chemical composition as mentioned before.

Microscopical counts and measurements of certain histological characters of the leaf blade of the fifth upper leaf on the main stem are presented in Table (8) and Figures (1) and (2).

As to salinity effect, 6000 ppm level caused more reduction in all the anatomical studied characters as compared to the control and other two restricted treatments. Table (8) and Figure 1 (a and b) showed that the number of stomata in upper epidermis of leaf blade per unit area was decreased at 6000 ppm of salinity as compared to the control. The results obtained are in harmony with the results of prior study on *Crotalaria retusa* and *Crotalaria verrucosa* [83].

Table 8 and Figure 2 (a and b) cleared that irrigation water salinity at 6000 ppm decreased leaf blade thickness compared with control. This could be attributed to the decrease in thickness of palisade tissue, spongy tissue and midrib. In this regard, Parida *et al.* [84] showed that the decrease in the palisade tissue thickness in the leaf of *Salvadora persica* at a salinity of 750 mM NaCl might be an adaptation of this plant to minimize the photosynthetic energy utilization under the saline condition.

It was obvious that the combined treatment of AM+R inoculum increased the number of stomata in upper epidermis of leaf blade than that of control Table 8 and Fig. 1 (a and c). This treatment exhibited positive effects on the structure of leaf blade with regard to the thickness of leaf blade, palisade tissue, spongy tissue and midrib as compared with the control Table 8 and Fig. 2 (a and c). The increased thickness of midrib was due to enlarged size of the most cells of the ground tissue as well as the increased number and size of vessels. The blade was more

Table 8: Mean values of some anatomical traits of leaf blade of the upper fifth leaf on the main stem of *Vachellia farnesiana* plant grown under sandy soil conditions as affected with 6000 ppm of salinity, AM+R inoculum and AM+R inoculum under 6000 ppm of salinity

	Treatments			
Characters	Untreated control	6000 ppm salinity	AM+R inoculum	(AM+R) inoculum + 6000 ppm salinity
Number of stomata per microscopic field area (unit area)	26	23	35	31
Thickness of blade (µm)	400	342	533	462
Thickness of palisade tissue (µm)	71	62	92	92
Thickness of spongy tissue (µm)	253	188	323	277
Thickness of midrib (µm)	1034	1016	1355	1140

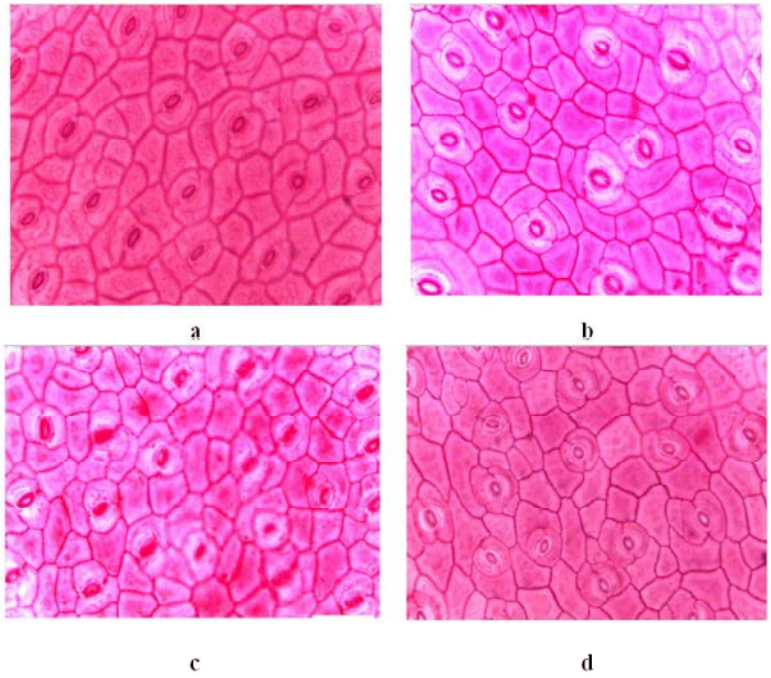

Fig. 1: An impression of upper leaf blade epidermis of the upper fifth leaf on the main stem of *Vachellia farnesiana* plant as treated with 6000 ppm of salinity, AM+R inoculum and AM+R inoculum under 6000 ppm of salinity (500 x)
a-Untreated
b-Treated (6000 ppm of salinity)
c-Treated (AM+R inoculum)
d-Treated (AM+R inoculum under 6000 ppm of salinity)

thickened in treated leaf than the control due to increasing thickness of mesophyll (palisade and spongy tissues). It is worthy to note that the combined treatment of AM+R inoculum which gave more positive effects on the studied characters as mentioned before, exhibited positive effects on leaf structure and the number of stomata. This might enhance the photosynthetic function of the treated leaves. Consequently, this might improve the growth of the treated plants under sandy soil condition. These obtained results are in agreement with the results of morphological traits and chemical composition.

As shown in Table 8 and Fig. 1 (a and d), inoculation with the combined treatment of AM+R inoculum under 6000 ppm of salinity increased the number of stomata on the upper surface of leaf blade as compared with the control. Table 8 and Figure 2 (a and d) cleared that this treatment exhibited prominent increments in thickness of leaf blade, palisade tissue, spongy tissue and midrib over than those of control. It can be concluded that applying AM+R inoculum to *Vachellia farnesiana* plants overcome the hazard effect of 6000 ppm salinity and improved tolerance of plants to irrigation water salinity under sandy soil condition.This might be attributed to the positive effect of inoculation treatment on osmotic adjustment achieved by uptake of inorganic ions from the soil solution as well as photosynthesis enhancement.

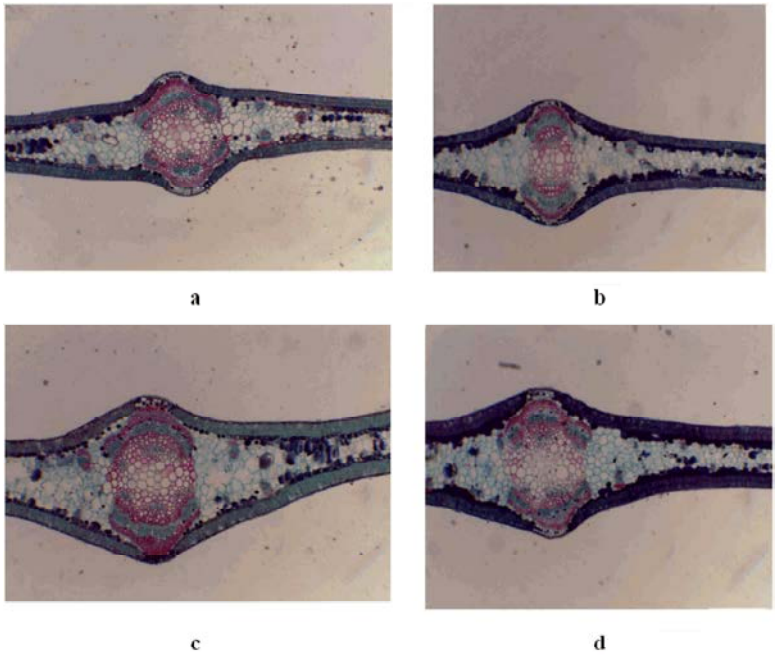

Fig. 2: Transverse sections in leaf blade of the upper fifth leaf on the main stem of *Vachellia farnesiana* plant as affected with 6000 ppm of salinity, AM+R inoculum and AM+R inoculum under 6000 ppm of salinity (50 x)

a-Untreated
b-Treated (6000 ppm of salinity)
c-Treated (AM+R inoculum)
d-Treated (AM+R inoculum under 6000 ppm of salinity)

DISCUSSION

The present findings revealed that inoculation with the combined treatment of arbuscular mycorrhiza fungi and *Sinorhizobium terangae* strains of bacteria counteracted the stress of irrigation water salinity (6000 ppm) increased *Vachellia farnesiana* plant vigor under saline conditions. The favorable effect of the inoculated plants related to increasing nitrogen content for porphyrin molecules in the cytochrome enzymes essential in photosynthesis. The increase in the contents of chlorophylls and cytochrome enzymes results in increasing photosynthesis [85]. Rhizobium improves plant growth and yield due to its role in atmospheric nitrogen fixation, phosphate and potassium solubilization and production of secondary metabolites such as plant hormone which ameliorate plant growth. Rhizobial inoculation caused the increase in percentages of nutrients due to the dehydrogenase of rhizobium forms carbonic acid leading to decreasing pH and increasing nutrient uptake and leading to better root and shoot growth. Rhizobacteria is responsible for preselecting nutrients and water flow to the roots of the plants as it forms bacterial exopolysaccharides, resulting in more extensive rhizosheaths that bind sodium cations [86]. *Rhizobium* produces cytokinins and/or gibberellins for inducing plant growth [80]. Oxidative damage to the plants treated with plant growth promoting rhizobacteria diminished due to increasing activities of superoxide dismutase, catalase, peroxidase and ascorbate peroxidase antioxidant enzymes [81, 85].

The present results showed that irrigation water salinity decreased plant growth and biomass production. It might affect the uptake of the essential nutrients causing the antagonism condition. A decrease in stomatal conduction is found to be associated with a decrease in net photosynthesis in several plant species under stress conditions [87]. It was noticed that there was a decline in transpiration rate due to salt stress in *Brassica* species [88]. Salinity mainly affects the plants by reducing the water potential of the soil, leading to deficiency of water available to plants. This decrease in water availability ultimately reduces the photosynthetic rate and hence the overall growth of the plant [89, 90]. Continued transport

of salt into transpiring leaves over a long period of time eventually results in higher Na^+ and Cl^- concentrations and senescence of older leaves, followed by a decline in photosynthesis leading to a decline in plant growth and yield [91]. Salinity induces ionic and osmotic imbalance at the cellular level which results in ion toxicity and osmotic stress [92].The accumulation of osmotic active material represents an osmotic adjustment which enables cells to absorb water and maintain turgidity for salinity tolerance [65]. Proline acts as an organic nitrogen reserve during salinity stress, it also functions as an antioxidant agent [93].The ability of plants to counteract stress depends on the level of K^+ available to the plant. K^+ is important to all the plant as a balancing charge and the plant must maintain a high potassium level to counterbalance the excess salt [75]. Some studies have shown that plants able to maintain a high level of potassium are also associated with salt tolerance [76].

CONCLUSION

From the results obtained, it can be concluded that *Vachellia farnesiana* plants can be grown in sandy soil using water with salt concentration up to 6000 ppm by inoculation with mixed spores of arbuscular mycorrhizal fungi from genera *Glomus*, *Gigaspora* and *Acaulospora* (450 spores/g sterilized peatmoss as a carrier) at the rate of 10g /pot (25 cm diameter) followed by two different *Sinorhizobium terangae* strains (R, 10^9 CFU/ml) at the rate of 10 ml/ pot.

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