Effect of Some Biofertilizers and Humic Acid Application on Olive Seedlings Growth under Irrigation with Saline Water

Mona, M. El-Shazly[1] and Wael M.Ghieth [2]

ABSTRACT

The present study was conducted to examine the effect of humic acid concentration (0, 0.5, 1 and 1.5 ml/L) and some of biofertilization treatments (control, *Azotobacter chroococcum*, Mycorrhizae (*Glomus macrocarbium*) and mix of *Azotobacter chroococcum* + Mycorrhizae on olive seedling which grown under three levels of saline water (2000, 3000 and 4000 ppm). this experiment was carried out during two successive seasons (2015 and 2016) on Olive seedlings Picual cultivars grown in El-Sheikh Zuwayid station, Desert Research Center North Sinai governorate, Egypt. Based on growth parameters data showed that salinity level (2000ppm) produced the highest significant parameters of the olive seedling; for seedling height, trunk diameter, Branch number., Leaf number., leaf length, leaf width, leaf area, and also the fresh and dry weight of shoot and root system. The lowest values were recorded for salinity level (4000 ppm through the two seasons. Salinity level at 2000 ppm gives the chance of growing plant to complete all of its physiological processes at a proper time than that of high concentration. Highest salinity concentration 4000 ppm caused a decline in all the studied parameters throughout both studied seasons. Increasing humic acid levels from 0.5 to 1.5 ml/L % increased significantly all studied parameters when compared with control (0) in the two studied seasons. Application of biofertilization treatments either singly or mixed enhanced growth and plant biomass of olive seedling under different salinity treatment. Mixed two types of biofertilzer had a significant effect on seedling growth than control and one type of biofertilizer treatments. In addition, Macronutrient content in olive seedling leaf positively affected with humic acid concentration and biofertilization treatments. Mixed biofertilization treatment resulted in higher values of soil microbiological properties, i.e. total microbial counts, Azotobacter densities, Mycorrhizal infection percentage, no. of mycorrhizal spores /gm, microbial enzymes in soil (Dehydrogenase, Nitrogenase and Phosphatase). It can be concluded that, to mitigate the negative impact of salinity of olive seedling we recommend to use humic acid (1.5 ml/L%) with the treatment of biofertilizer (Mycorrhiza and *Azotobacter chroococcum*).

Keywords: Olive seedling, Salinity, Humic acid, Biofertilzer, *Azotobacter*, Mycorrhizae.

INTRODUCTION

Olive (*Olea europaea L*) is considered as a moderately salt tolerant fruit crop, it is better than citrus, but less than Palm tree, irrigation with saline water can be harmful to many of olive tree with negative impacts on growth and behavior. In most coastal Mediterranean areas, in which olive is cultivated, the increased need for good quality water for human utilization limits the use of water for irrigation. Therefore, in those areas, large quantities of low-quality water are available especially saline water, which should be used to replenish irrigation requirements. Salinization of soils and waters is one of the world's most serious environmental problems in agriculture; it is limiting crop growth and productivity especially in arid and semi-arid regions (Sepaskhah and Yarami, 2010). Toxicity of Na^+ in metabolic processes results from its ability to compete with $K^+$for binding sites and to inactivate enzymes and essential cellular functions and, consequently, crops growing in saline soils may suffer the dual injury of Na^+ toxicity and low K^+ concentrations (Munns and Tester, 2008).

Humic acids (HA) are the most active components of soil and compost organic matter, stimulate plant growth and consequently yield by acting on mechanisms involved in cell respiration, photosynthesis, protein synthesis, water and nutrient uptake, enzyme activities (Chen *et al*.,2004). In particular, optimal concentrations able to affect and stimulate plant growth have been generally found in the range of 50-300 mg/ L, but positive effects have been also exerted by lower concentrations (Chen *et al*., 2004). A distinction on the effects of humic acids should be made between indirect and direct effects on plants growth. Indirect effects are mainly exerted through properties such as enrichment in soil nutrients, increase of microbial population, higher cation exchange capacity, improvement of soil structure; whereas direct effects are various biochemical actions exerted at the cell wall, membrane or cytoplasm and mainly of hormonal nature (Varanini and Penton, 2001; Chen *et al*., 2004). Magdi *et al*.,(2011) reported that bio-fertigation of microbial inocula and humic substances could be used as a complementary for

DOI: 10.21608/ASEJAIQJSAE.2019.33657

[1]Soil Fertility and Microbiology Department, Desert Research Center

[2]Pomology unit, Department of plant production, Desert Research Center

Received April 14, 2019, Accepted May 21, 2019

mineral fertilizers to improve yield and quality of cowpea under sandy soil conditions which protect the environment chemical pollution and its harmful effect on human and animal health. A foliar application of HA increased the vegetative growth of olive cuttings (Hartwigsen and Evansmicheal, 2000; Muscolo and Sidari, 2007; Schmidt *et al*. 2007; Zandonadi *et al*. 2007).

Biofertilizers are biological products containing living microorganisms that, when applied to seed, plant surfaces, or soil, promote growth by several mechanisms such as increasing the supply of nutrients, increasing root biomass or root area, and increasing nutrient uptake capacity of the plant (Vessey 2003). Biofertilizers can be used as complements to mineral fertilizers (Canbolat *et al*. 2006). Microbial inoculants mainly include free-living bacteria, fungi, and arbuscular mycorrhizal fungi (AMF) (Berg 2009; Dodd and Ruiz-Lozano 2012; Vessey 2003) that were isolated from a variety of environments including soil, plants, plant residues, water, and composted manures.

Arbuscular Mtycorrhizal Fungi (AMF) is known to enhance plant establishment and drought tolerance (Querejeta *et al*. 2003) by various mechanisms including (a) improved water uptake, by which AMF effectively extend plant roots making the uptake of water much more efficient; (b) better mineral nutrition, especially phosphorus, as a consequence of effectively extending roots; (c) alterations in root architecture;(d) modification of some physiological and enzymatic activities, especially those involved in plant antioxidative responses; and (e) induction of the plant hormone Abscisic acid (ABA), which can play an important role in mediating some plant responses to different stresses including drought (Gamalero *et al.* 2002). Under these conditions, AMF enhanced root surface area and promoted dense root growth, resulting in improved drought tolerance. Moreover, plants colonized by AMF were able to maintain higher water use efficiency, and growth was increased at a faster rate when irrigation was restored. Such adjustment of osmotic potential is one of the most important factors for plant survival under drought conditions. In addition, AMF may affect plant water potential by modification of soil structure.

Hyphae of AMF can improve soil structure by binding soil particles and producing glomalin, an insoluble gluelike substance (Augé 2001). AMF may also play a role in the protection of roots from heavy metal toxicity by mediating interactions between metals and plant roots (Leyval *et al*. 1997).

The beneficial effect of symbiotic nitrogen fixer *Azotobacter chroococcum* as free-living N_2- fixing is attributed to fix atmospheric nitrogen, synthesis of phytohormones and vitamins, inhibiting plant ethylene synthesis, enhancing stress resistance and improving nutrient uptake (Massoud *et al*., 2013).

Co-inoculation of AMF and Plant growth regulating rhizobacteria (PGPR) is also a promising strategy to increase plant tolerance to salinity and drought. It was reported that co-inoculation of the AMF *Glomus mosseae* and *G. intraradices* and PGPR *Bacillus spp*. on lettuce increased plant growth, photosynthetic rate, water use efficiency, and stomatal conductance after drought stress. The effect of the co-inoculation was better than inoculation with only AMF or *Bacillus spp*. Furthermore, *Bacillus spp*. inoculation also improved AMF colonization and growth (Vivas *et al*. 2003). The objectives of this study were to evaluate the effect of the application of humic acid and biofertilization on olive seedlings under three levels of saline water (2000, 3000 and 4000 ppm).

MATERIALS AND METHODS

A field experiment was conducted at El-Sheikh Zuwayid research station, Desert Research Center (DRC), North Sinai Governorate for two growing seasons (2015 and 2016) on olive seedlings to study the effect of Biofertilization treatments and humic acid concentrations on the growth of olive seedling under three different salinity levels. The experiment was laid out in a split split plot design with three replications.

Three salinity levels were used (2000, 3000 and 4000 ppm) which obtained from El-Sheikh Zuwayid research station wells, four concentration of organic humic acid (0, 0.5, 1 and 1.5 ml in 1 Liter of water) were applied as foliar spray with four biofertilization treatments (control, *Azotobacter chroococcum*, Mycorrhizae *(Glomus Macrocarbium*) and mix of Mycorrhiza and *Azotobacter chroococcum*). The seedling was about 1-year-old (Picual cultivar) planted at 6 × 6 m apart grown in sandy soil, under drip irrigation system and nearly uniform in shape and received the common horticultural practices.

The chemical and physical characteristics of the experimental soil are presented in Table. 1. The rate of fertilizers added were 10 m^3 chicken manure per feddan, 500 grams of superphosphate, 250 grams ammonium sulfate and 250 grams potassium sulfate per tree were applied at the time of the winter service followed by adding 500 ml (5×10^6 cfu) of pure active culture of *Azotobacter chroococcum* and 100g of crude inoculum of *Glomus macrocarpum* (≈ 10 spores/g) singly and mixed to the root zone of olive trees.

Table 1. The main physical and chemical characteristics of the experimental soil of El-sheikh Zuwayid research station.

Physical parameters	Very coarse sand %	Coarse sand %	Medium sand %	Fine sand %	Very fine sand	Silt and clay %	Soil texture
	0.31	1.91	40.05	53.52	2.44	1.75	Sandy soil

Chemical parameters	pH	E.C. dS/m	O.C.%	T.N. %	Water soluble cations meq/l				Anions meq/l			
					Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	$HCO3^{--}$	Cl^{-}	SO_4^{--}
	7.69	1.71	0.053	0.0016	7.94	2.78	1.62	3.81	4.86	-	5.62	5.69

These microbial and humic acid spray treatments were applied at three growth stages before spring growth cycle (March), after 2 months from the first treatment (May) and after 2 months from the second treatment (July) in the two studied seasons.

Preparation of microbial inoculate

The heavy cell suspension of *Azotobacter chrocchocum* was obtained by growing *A.chroococcum* on Ashby´s media for 7 days at 28±2˚C. The proliferation of mycorrhizal spores was carried out by pot culture; mycorhizael spores were obtained by wet-sieving method (Gerdemann and Nicolson, 1963). The isolated spores were proliferated with the barley (*Hordeum vulgare*) as a host plant in the pots for production of mycorrhizal inoculum. Then the rhizosphere soil till depth 15 cm from the roots and roots were used as a mycorrhiza crude inocula after 10 days from cutting the vegetative parts.

Sampling and determinations

Growth and seedling weight parameters

In late October, tree height (cm) was measured for each season also trunk diameter, Branch number, leaves number, Leaves length, leaf width and leaf area were determined for two seasons according to Ghieth, (2009). Fresh and dry weight for a seedling shoot and root system were determined at the second season.

Determination of Macronutrients (NPK)

Leaves samples were collected at the end of June 2016 and then dried at 70º C in a hot air oven for 3 hrs. The dried samples were ground and then digested for nitrogen, phosphorus, potassium analyses. Nitrogen, Phosphorus and Potassium were measured according to Page *et al.* (1982).

Microbiological analysis

Rhizosphere soil samples were collected to determine the microbiological parameters, the root hairs collected for detection the percentage of mycorrhizal colonization.

Total microbial counts were determined according to Nautiyal (1999), *Azotobacter* densities were determined by using the most probable number (MPN) method after incubating the tubes at 28 + 2 ˚C for 10 days on modified Ashby's medium (Becking , 2006). Total actinomycetes count was determined on starch nitrate medium (Waksman & lechevalier, 1962).

The mycorrhizal colonization in roots was estimated by the gridlines intersect method of Giovannetti & Mossa, (1980).

Statistical analysis

Data were subjected to statistical analysis using the method described by Snedecor and Cochran (1990). The least significant difference (L.S.D.) was used to differentiate means according to (Waller and Duncan, 1969).

RESULTS AND DISCUSSION

Plant growth characteristics

Data in Table 2 indicates that in the two growing seasons, seedling height, trunk diameter and branches number measurements exhibited significant differences for different salinity levels. The highest increases were obtained with salinity level (2000 ppm) being 83.59 cm,0.97 cm and 16.89 respectively , while 4000 ppm gave the lowest values being 64.69 cm, 0.85 cm and 11.99 for height , trunk diameter and branches number in the first season while the same parameters gave 123.4 cm,1.24 cm and 13.01 with salinity level (2000 ppm) while it gave 101.36 cm,1.1 cm and 11.43 respectively with salinity level (4000 ppm) in the second season. Salinity stress reduces height by nearly 21.7 % for 4000 ppm compared with 2000 ppm at second season; this may be due to the negative impact of salinity on plant growth. Salinity stress depresses plant growth and development at different physiological levels. The mechanism by which salt stress damage plants are still a discussing matter due to the very complex nature of the salt stress in plants (Zhu, 2001).

Table 2. Effect of water irrigation Salinity, humic acid concentration and biofertilization treatments on height, trunk diameter and branch no. of olive seedling in the two growing seasons.

Treatment	1st season			2nd season		
	Height (cm)	Trunk Diameter (cm)	Branch No.	Height (cm)	Trunk Diameter (cm)	Branch No.
Salinity(ppm)						
2000	83.59	0.97	16.89	123.34	1.24	13.01
3000	72.24	0.86	13.80	112.70	1.16	12.13
4000	64.96	0.85	11.99	101.36	1.10	11.43
L.S.D at 5%	0.24	0.73	0.30	0.36	0.02	0.26
Humic acid (ml/L)						
0	56.56	0.84	11.67	96.71	1.08	10.03
0.5	70.00	0.85	13.65	108.36	1.11	11.24
1.0	77.70	0.89	14.66	115.36	1.18	12.76
1.5	90.72	0.91	16.92	129.36	1.30	14.74
L.S.D at 5%	0.36	0.01	0.33	0.23	0.03	0.38
Biofertilization treatments						
Control	37.80	0.84	11.08	66.50	1.01	10.31
A.chrococcum	74.62	0.86	13.07	117.88	1.13	12.17
G.macrocarbium	83.44	0.90	15.40	125.72	1.23	11.94
Mixture	99.26	0.92	17.23	139.96	1.30	14.35
L.S.D at 5%	0.41	0.15	0.66	0.37	0.03	0.21

Among different rates of humic acid, there were significant variations at the level (p> 0.05). This may be due to humic acid is known to promote nutrient uptake as a chelating agent and improves vegetative characteristics, nutritional status and leaf pigments (Eissa *et al*, 2007).

The highest concentration of humic acid recorded the highest increment for height, trunk diameter and branches number of Olive seedling being 60.39, 8.33 and 44.98 % of increase than control at first season and 33.76 ,20.37 and 46.95 % of increase than control at second season respectively. The results are in agreement with Zandonadi *et al.* 2007 they reported that a foliar application of humic acid increased the vegetative growth of olive cuttings. In particular, optimal concentrations able to affect and stimulate plant growth have been generally found in the range of 50-300 mg L^{-1}, but positive effects have been also exerted by lower concentrations (Chen *et al.,* 2004).

Biofertilization treatments showed a stimulating effect on different growth parameters either alone or in combination. Mixed treatments exerted percentage increase height, trunk diameter and branches number by 162, 9.52 and 55.50 % at a 1st season, and by 110.64, 28.71 and 39.18 % at a 2nd season, respectively. For single treatments, mycorrhizae give the highest value compared to Azotobacter. Such increases by the different biofertilization treatments emphasize the fact that biofertilization stimulates vegetative growth through different mechanisms like improve plant metabolic activity, enhance nutrient uptake, secretion of plant growth promoting substances like hormones, vitamins, nitrogen fixation, organic acid production (Muhammed *et al*., 2012).

Table 3, clearly showed that humic acid (HA) treatments at a concentration (1.5ml/L) with Mixed Biofertilization treatments and first salinity level significantly (p> 0.05) increased all the studied parameters; i.e., seedling height, trunk diameter and branch no. than untreated control during1st and 2nd season. The positive influence of HA on plant growth and productivity, which seems to be concentration specific, could be mainly due to the hormone-like activity of HA through its involvement in cell respiration, photosynthesis, oxidative phosphorylation, protein synthesis, and various enzymatic reactions (Muscolo and Sidari 2007). The highest values of plant height, trunk diameter and branches number (134.40 , 1.06 and18.66 at 1st season) and (177.80, 1.76 and 24.74) at 2nd season) respectively, were recorded at the concentration of 1.5 HA. These results may be due to the role of HA in enhancing some physiological and biochemical aspects (Schmidt *et al*. 2007).

Table 3. Interaction effect of water irrigation Salinity, humic acid and biofertilization treatments on some of the tree parameters of olive seedling in the two growing seasons.

Salinity ppm	Humic ml/L	Biofertilization treatments	1st season			2nd season		
			Height (cm)	Trunk Diameter(cm)	Branch. No	Height (cm)	Trunk Diameter(cm)	Branch No.
2000	0	Control	32.66	0.81	9.38	61.14	0.95	10.74
		A.chrococcum	65.80	0.84	9.80	108.26	1.12	12.46
		G.macrocarbium	70.00	0.87	10.74	112.94	1.23	15.86
		Mixture	71.40	0.99	12.18	111.54	1.25	16.34
	0.5	Control	40.60	0.83	10.26	62.54	0.94	12.14
		A.chrococcum	81.20	0.84	11.66	126.46	1.13	14.94
		G.macrocarbium	91.98	0.94	12.18	134.86	1.26	17.74
		Mixture	105.98	1.02	13.58	149.80	1.27	17.74
	1	Control	46.20	0.88	11.66	65.38	1.05	13.06
		A.chrococcum	89.60	0.88	13.06	138.18	1.25	17.26
		G.macrocarbium	99.86	0.95	13.58	142.80	1.39	20.06
		Mixture	123.66	1.05	15.86	169.40	1.30	19.14
	1.5	Control	50.40	0.90	13.58	78.40	1.16	13.11
		A.chrococcum	107.80	0.95	15.86	149.80	1.30	19.60
		G.macrocarbium	99.86	0.99	16.38	116.66	1.44	24.26
		Mixture	134.40	1.06	18.66	177.80	1.76	24.74
3000	0	Control	29.40	0.80	8.40	62.06	0.88	9.34
		A.chrococcum	58.80	0.81	10.26	99.86	1.05	10.26
		G.macrocarbium	67.20	0.83	9.80	109.20	1.15	13.06
		Mixture	64.86	0.85	11.66	110.18	1.23	13.54
	0.5	Control	35.00	0.81	9.80	63.98	0.92	10.26
		A.chrococcum	67.20	0.83	11.20	109.20	1.11	11.66
		G.macrocarbium	77.00	0.87	10.78	132.06	1.23	13.54
		Mixture	98.00	0.88	13.06	148.86	1.25	16.80
	1	Control	39.66	0.85	10.78	67.66	0.97	11.20
		A.chrococcum	77.00	0.84	12.60	119.46	1.12	13.06
		G.macrocarbium	82.60	0.90	12.18	124.60	1.26	14.46
		Mixture	106.40	0.92	15.40	147.46	1.26	20.54
	1.5	Control	44.80	0.87	11.66	74.66	1.01	11.66
		A.chrococcum	95.20	0.92	14.46	137.66	1.15	14.46
		G.macrocarbium	96.60	0.94	14.00	138.18	1.27	16.34
		Mixture	124.18	0.95	18.20	171.26	1.64	22.40
4000	0	Control	28.46	0.78	7.94	56.46	0.87	7.94
		A.chrococcum	56.98	0.78	9.80	97.06	0.99	9.80
		G.macrocarbium	64.40	0.81	8.86	106.40	1.08	10.74
		Mixture	66.78	0.84	9.34	107.34	1.11	11.20
	0.5	Control	30.80	0.80	8.86	58.34	0.91	9.80
		A.chrococcum	61.60	0.80	10.74	105.00	1.06	11.20
		G.macrocarbium	69.06	0.83	9.80	111.06	1.11	11.66
		Mixture	81.66	0.87	12.60	111.06	1.13	13.54
	1	Control	33.60	0.83	10.26	59.26	0.95	10.26
		A.chrococcum	64.40	0.81	12.14	110.14	1.11	12.60
		G.macrocarbium	72.24	0.85	11.66	113.86	1.12	13.06
		Mixture	98.00	0.90	14.00	124.60	1.27	14.00
	1.5	Control	39.20	0.83	10.74	70.46	0.99	10.74
		A.chrococcum	69.02	0.90	13.54	112.46	1.11	13.54
		G.macrocarbium	85.40	0.91	13.54	127.40	1.23	15.86
		Mixture	117.60	0.94	15.40	149.34	1.50	16.34
L.S.D at 5%			0.97	0.069	1.36	0.10	0.71	1.21

In addition, PGPR (plant growth promoting rhizobacteria) can improve plant growth, plant nutrition, root growth pattern, plant competitiveness and responses to external stress factors. PGPR have also been shown to induce systematic resistance (ISR) to fungal, bacterial, and viral pathogens in various crops such as bean, tomato, radish, and tobacco (Glick,1995).

Table 4 clearly showed that leaf measurements (leaf number, leaf length, leaf width and leaf area) negatively affected by increasing salinity during the two seasons. Different humic acid concentration positively affected leaf measurements while the highest concentration recorded the highest value for all measurements. While biofertilization treatments significantly affected leaf measurements especially, mixed biofertilization treatments (mycorrhizae+Azotobacter) recorded highest values being 167.31,6.18 cm,1.37 cm and 6.37 cm^2 for leaf number, leaf length, leaf width and leaf area respectively at first season compared to single treatments and control while, it recorded 381.12, 6.22 cm,1.39 cm and 6.33 cm^2 at second season respectively.

As for interaction effect data in Table 5 demonstrated that biofertilization treatments improved leaf measurements (leaf number, leaf length, leaf width and leaf area) under different salinity level and different humic acid concentration. The treatment of a mixture with the lower level of salinity and humic acid (1.5 ml/L) achieved the highest significant leaf measurements (274.70,6.4 cm, 1.5 cm and 6.9 cm^2) in the first and (757.5, 6.77 cm, 1.53 cm7.1 cm^2) in the second season, respectively. Represented results clearly revealed that biofertilization treatments with humic acid concentration reduce the negative effect of salinity.

The obtained results in agreement with Muscolo and Sidari 2007 they reported the positive influence of HA on plant growth and productivity, which seems to be concentration specific, could be mainly due to the hormone-like activity of HA through its involvement in cell respiration, photosynthesis, oxidative phosphorylation, protein synthesis, and various enzymatic reactions. Also, Rojas et al., 2012 showed that Inoculation with *Azotobacter* strains has been shown to have generally positive effects under saline stress by facilitating uptake of K^+ and exclusion of Na^+ as well as increasing phosphorous and nitrogen availability.

Tables 6 and 7 showed that, the highest olive fresh and dry weight was attained by salinity level 2000 so, increasing salinity level decease fresh and dry weights of Olive seedling in addition ,fresh and dry weight of seedling was also affected by different humic acid concentration increasing humic acid concentration lead to increasing both fresh and dry weight for both shoot and root system in the second season.

Table 4. Specific effect of water irrigation Salinity, humic acid concentration and biofertilization treatments on Leaf measurements of olive seedling in the two growing seasons.

Treatment levels	1st season				2nd season			
	Leaf No.	Leaf length	Leaf width	Leaf area	Leaf No.	Leaf length	Leaf width	Leaf area
Salinity(ppm)								
2000	128.1	6.08	1.3	6.3	294.00	6.13	1.38	6.33
3000	106.5	5.97	1.26	6.2	245.20	6.01	1.3	6.16
4000	84.2	5.79	1.25	5.92	183.20	5.8	1.27	5.89
L.S.D at 5%	0.985	0.0104	0.0227	0.0163	1.02	0.0196	0.0169	0.0255
Humic(ml/L)								
0	84.61	5.65	1.22	5.72	183.62	5.71	1.296	5.72
0.5	100.25	5.95	1.234	6.1	226.40	5.95	1.3	6.03
1.0	113.22	6.03	1.28	6.25	257.60	6.03	1.32	6.27
1.5	126.97	6.2	1.32	6.5	295.60	6.23	1.34	5.5
L.S.D at 5%	0.995	0.0177	0.0204	0.0243	1.09	0.0232	0.0131	0.0164
Biofertilization treatments								
Control	75.8	5.51	1.2	5.7	154.88	5.59	1.27	5.85
A.chrococcum	83.92	6.02	1.25	6.14	189.00	6.04	1.28	6.14
G.macrocarbium	98.1	6.08	1.25	6.34	238.12	6.11	1.31	6.2
Mixture	167.31	6.18	1.37	6.37	381.12	6.22	1.39	6.33
L.S.D at 5%	1.13	0.0161	0.0124	0.0202	0.52	0.0176	0.0177	0.0168

Table 5. Interaction effect of water irrigation Salinity, humic acid and biofertilization treatments on Leaf measurements of olive seedling in the two growing seasons.

Salinity ppm	Humic ml/L	Biofertilization treatments	1st season				2nd season			
			Leaf No.	Leaf len.	Leaf wid.	Leaf area	Leaf No.	Leaf len.	Leaf wid.	Leaf area
2000	0	Control	72.00	5.3	1.2	5.6	188.3	5.3	1.31	5.66
		A.chrococcum	78.00	6	1.2	5.9	209.3	5.8	1.37	5.7
		G.macrocarbium	105.00	6	1.2	5.9	332.5	6.1	1.34	5.97
		Mixture	133.70	6.1	1.4	6	390.8	6.07	1.37	5.93
	0.5	Control	78.00	6	1.2	5.7	188.3	5.7	1.31	5.99
		A.chrococcum	81.70	6.1	1.2	6.4	236.8	6.13	1.33	6.2
		G.macrocarbium	117.00	6.1	1.3	6.4	365.0	6.17	1.35	6.25
		Mixture	204.70	6.2	1.4	6.3	550.8	6.13	1.47	6.37
	1	Control	83.30	6.1	1.3	5.8	210.0	5.74	1.36	6.09
		A.chrococcum	105.70	6.2	1.3	6.6	303.3	6.27	1.3	6.61
		G.macrocarbium	138.70	6.3	1.3	6.8	443.3	6.4	1.37	6.65
		Mixture	228.70	6.3	1.4	6.6	650.8	6.2	1.5	6.67
	1.5	Control	81.70	6.3	1.3	6.1	219.3	5.95	1.4	6.27
		A.chrococcum	117.35	6.4	1.3	6.8	338.3	6.53	1.29	6.92
		G.macrocarbium	150.35	6.4	1.3	6.9	496.8	6.53	1.43	6.93
		Mixture	274.70	6.4	1.5	6.9	757.5	6.77	1.53	7.1
3000	0	Control	68.35	5.2	1.2	5.4	178.3	5.2	1.25	5.37
		A.chrococcum	76.65	5.8	1.3	5.8	188.3	5.83	1.29	5.87
		G.macrocarbium	79.35	5.9	1.1	5.9	221.8	5.93	1.3	5.94
		Mixture	111.65	5.9	1.3	5.9	303.3	5.93	1.36	5.81
	0.5	Control	73.00	5.7	1.2	5.7	182.5	5.54	1.26	5.92
		A.chrococcum	79.35	6	1.2	6.1	220.0	5.87	1.28	6.07
		G.macrocarbium	82.00	6.1	1.2	6.3	224.3	6.13	1.29	6.15
		Mixture	173.70	6.2	1.3	6.1	590.0	6.3	1.31	6.17
	1	Control	78.70	5.7	1.3	5.8	180.8	5.69	1.28	6.02
		A.chrococcum	81.30	6.1	1.2	6.3	235.0	6.1	1.27	6.27
		G.macrocarbium	87.30	6.2	1.2	6.7	252.5	6.13	1.28	6.28
		Mixture	199.30	6.2	1.4	6.4	605.8	6.47	1.37	6.57
	1.5	Control	89.70	5.9	1.3	6	212.5	5.93	1.29	6.25
		A.chrococcum	92.30	6.2	1.3	6.5	277.5	6.27	1.26	6.5
		G.macrocarbium	95.70	6.2	1.3	6.9	310.0	6.27	1.33	6.53
		Mixture	251.30	6.3	1.4	6.9	720.8	6.57	1.4	6.87
4000	0	Control	62.00	5	1.1	5.3	167.5	4.9	1.17	5.35
		A.chrococcum	64.00	5.7	1.2	5.5	180.8	5.73	1.24	5.53
		G.macrocarbium	71.70	5.8	1.2	5.7	203.3	5.8	1.23	5.75
		Mixture	94.00	5.9	1.3	5.8	162.5	5.9	1.33	5.79
	0.5	Control	72.70	5.2	1.2	5.6	190.0	5.44	1.25	5.5
		A.chrococcum	72.70	5.8	1.2	5.8	191.8	5.87	1.23	5.83
		G.macrocarbium	75.70	5.9	1.2	6	210.8	5.93	1.24	5.89
		Mixture	93.70	6.1	1.3	6.1	244.3	6.19	1.29	6.05
	1	Control	76.70	5.3	1.2	5.6	200.8	5.45	1.26	5.81
		A.chrococcum	78.00	6	1.2	6	217.5	6.13	1.2	6.07
		G.macrocarbium	82.00	5.9	1.2	6.2	230.8	5.87	1.27	5.93
		Mixture	119.30	6.2	1.4	6.4	331.8	6.23	1.37	6.2
	1.5	Control	77.30	5.4	1.2	5.9	204.3	5.38	1.25	6.02
		A.chrococcum	86.00	6	1.3	6.1	236.8	6.17	1.18	6.1
		G.macrocarbium	92.30	6.2	1.3	6.2	280.8	6.23	1.32	6.03
		Mixture	124.00	6.3	1.4	6.8	393.3	6.33	1.4	6.43
L.S.D at 5%			1.939	0.064	0.97	0.064	0.043	0.07	2.30	0.0583

Table 6. A specific effect of water irrigation Salinity, humic acid concentration and biofertilization treatments on a shoot and root system of olive seedling in the 2nd season.

Treatment levels	2nd season			
	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Salinity(ppm)				
2000	208.08	77.80	173.60	66.88
3000	191.88	73.32	158.48	60.36
4000	178.92	70.20	145.36	56.08
L.S.D at 5%	1.02	0.06	0.95	0.43
Humic(ml/L)				
0	177.36	66.92	142.92	53.40
0.5	185.44	69.04	152.96	56.56
1.0	198.80	78.64	164.12	65.36
1.5	210.40	80.48	176.64	69.00
L.S.D at 5%	0.94	0.08	1.30	0.49
Biofertilization treatments				
Control	136.02	59.16	102.40	46.20
A.chrococcum	195.72	69.36	162.40	56.80
G.macrocarbium	208.80	78.44	175.12	66.40
Mixture	231.60	88.12	196.84	75.00
L.S.D at 5%	0.98	0.18	1.01	0.49

As shown in Table 6 biofertilization treatments greatly affected the fresh and dry weight of seedling shoot and root system, Mixed treatments followed by single treatment with Myccorhizae than azotobacter recorded the highest value compared to control (without inoculation).

Concerning interaction effect on different salinity levels, three humic acid concentration and biofertilization treatments obtained results in Tables 7 clearly showed that, humic acid with Biofertilization treatment with different salinity levels enhanced fresh and dry weight of Olive seedling shoot and root system in the second season, the highest increment was recorded by humic acid at concentration(1.5) with mixed treatment at salinity level 2000 being 277.72 g and 106 g for fresh and dry shoot system weight respectively . While fresh and dry root system weight recorded 240.28 g and 91.72 g in the second season respectively.

Stimulating effect clearly appears between humic acid application and biofertilization treatments to mitigate salinity effects as shown by Magdi *et al.*, (2011) they reported that biofertilization and humic acid could be used as a complementary for mineral fertilizers to improve yield and quality of cowpea.

Macronutrients

Table 8 indicated that the highest values of N, P and K contents were attained by salinity level 2000 so, increasing salinity level decrease N, P and K in Olive leaves, in addition, Macronutrients were also affected by different humic acid concentration increasing humic acid concentration lead to increasing the values of NPK in both studied seasons.

As shown in Table 8 biofertilization treatments greatly affected of the values of NPK, Mixed treatments followed by single treatment with Myccorhizae, azotobacter recorded the highest values in comparison with control (without inoculation) in both studied seasons.

As shown in Table 9 the interaction between mixed biofertilization and HA gave synergistic with the lowest level of salinity gave the highest values of N, P and K being 208, 15.7 and 286 ppm in the first season and 214,15.68 and 291 in the second season, respectively.

Treatment with *A.chroococcum* and *G.macrocarbium* mitigated the adverse effect of salinity where Olive seedlings were able to grow and survive with the majority of treatments. Application of HA (0.5, 1 and 1.5 ml/L) alleviate the adverse effect of salinity on Olive seedlings. Enhancement of leaves contents of nutrients using humic acid had been noticed to be due to increased nutrients uptake such as N, P, K, Ca, Mg, Fe, Zn and Cu David et al., 1994.

Table 7. Interaction effect of water irrigation Salinity, humic acid concentration and biofertilization treatments on shoot and root system weight of olive seedling in the 2nd season

Salinity	Humic	Biofertilizers	2nd season			
			Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
2000	0	Control	126.28	48.2	92.52	35.32
		A.chrococcum	186.52	71.2	154.28	58.88
		G.macrocarbium	197.72	75.48	165.72	63.48
		Mixture	235.08	89.6	193.72	73.96
	0.5	Control	151.48	57.84	103.72	39.6
		A.chrococcum	193.88	74	165.32	63.12
		G.macrocarbium	206	78.64	176	67.16
		Mixture	249.6	95.28	216.8	82.76
	1	Control	198.4	75.6	113.08	43.16
		A.chrococcum	211.32	80.68	177.6	67.8
		G.macrocarbium	222.52	84.92	194.68	74.32
		Mixture	266.4	101.68	234	89.32
	1.5	Control	151.32	57.76	134.68	51.4
		A.chrococcum	151.72	57.92	194.52	74.24
		G.macrocarbium	234.68	89.6	202.4	77.24
		Mixture	277.72	106	240.28	91.72
3000	0	Control	118.12	45.08	81.72	31.2
		A.chrococcum	190.12	72.56	146.8	56.04
		G.macrocarbium	190.8	72.84	154.52	58.96
		Mixture	210.4	80.32	179.48	68.52
	0.5	Control	126.12	48.12	94.68	36.12
		A.chrococcum	193.6	73.92	164.28	62.72
		G.macrocarbium	199.2	76.04	168.92	64.48
		Mixture	225.32	86	189.88	72.48
	1	Control	128.12	48.92	103.08	39.36
		A.chrococcum	201.72	77	166.12	63.4
		G.macrocarbium	210.8	80.48	183.2	69.92
		Mixture	243.6	92.96	201.6	76.96
	1.5	Control	146.4	55.88	113.72	43.4
		A.chrococcum	208	79.4	178.52	68.16
		G.macrocarbium	229.72	87.68	194.28	74.16
		Mixture	248.12	94.72	208.28	79.52
4000	0	Control	114.4	43.68	74.28	28.36
		A.chrococcum	164.12	62.64	128.12	48.92
		G.macrocarbium	182.68	69.72	150.8	57.56
		Mixture	187.08	71.4	157.2	60
	0.5	Control	114.8	43.8	86.68	33.08
		A.chrococcum	170.12	48.2	143.32	35.32
		G.macrocarbium	197.6	71.2	159.48	58.88
		Mixture	198	75.48	166.4	63.28
	1	Control	131.2	89.72	98.12	73.96
		A.chrococcum	195.32	57.84	153.48	39.6
		G.macrocarbium	211.72	74	168.68	63.12
		Mixture	202.28	78.64	171.72	67.16
	1.5	Control	137.32	95.28	103.08	82.76
		A.chrococcum	202.12	75.72	166.92	43.16
		G.macrocarbium	221.48	80.68	182.8	67.8
		Mixture	233.88	84.92	186.8	74.32
		L.S.D at 5%	3.4116	0.3016	1.652	1.6876

Table 8. Specific effects of salinity level, humic acid and biofertilization on macronutrients content at Olive rhizosphere at 2nd seasons.

treatment	1st season			2nd season		
	N ppm	P ppm	K ppm	N ppm	P ppm	K ppm
Salinity (ppm)						
2000	178.4	13.3	250.8	181.7	13.4	256.3
3000	174.1	12.6	245.6	177.9	13.1	249.8
4000	171.2	12.6	233.9	174.4	12.74	239.1
L.S.D at 5%	0.163	0.0407	0.149	0.615	0.068	0.1883
Humic (ml/L)						
0	170	12.7	235.3	173.1	12.84	238.8
0.5	173.3	12.8	239.7	177	13.1	245.8
1.0	176.3	13	244.6	179.5	13.12	250.5
1.5	178.6	13.12	254.2	182.4	13.25	258.56
L.S.D at 5%	0.158	0.049	0.194	0.7013	0.0137	0.2576
Biofertilization treatments						
Control	155.92	11.11	229.8	158	11.23	232.4
A.chrococcum	185.33	11.98	237.6	188.8	12.1	243.4
G.macrocarbium	163.1	13.93	248.1	166.2	14.2	253.3
Mixture	193.9	14.6	258.4	199	14.8	264.5
L.S.D at 5%	0.09	0.047	0.1268	0.556	0.115	0.2546

These results agree with those of Pellerin et al., 2007 who concluded that mycorrhiza is capable of taking up, translocation and transferring water and nutrients because of the enlarged surface area of the roots zone. In the same trend, many investigators demonstrated that the positive effect of dual inoculation with N_2-fixer and P-solubilizer.

In the same trend, many investigators demonstrated that the positive effect of dual inoculation with N2-fixer and P-solubilizer.

Table 10 showed that the microbial determination (Total microbial counts, Azotobacter densities, mycorrhizal infection % and Mycorrhizal spores) at rhizosphere of inoculated plants were recorded significant increases with salinity level 2000 and decrease with increasing salinity, HA concentrations 1.5% recorded the highest value for microbial determination. For Biofertilization treatments it was found that microbial determination recorded the highest value with mixed inoculation treatment compared with control These results incompatible with the finding of (El-Wakeil and El-Sebai, 2007) who reported that total microbial count was higher significantly in mixed inoculant's strains than in single inoculant.

It was clear from the data represented in Table 10 that inoculation with A.chroococcum and G.macrocarbium stimulated the activity and growth Total microbial counts, Azotobacter densities, mycorrhizal infection percentage and Mycorrhizal spores.

The interaction between salinity, humic acid concentrations and Biofertilization treatments were represented in Table 11 The highest total microbial counts were associated with first salinity level (2000 ppm), humic acid 1.5 % and mixed treatment (*A.chroococcum* and *G.macrocarpium*) being 190 and 211×105 cfu/g dry soil at first and second seasons respectively. These results are compatible with those obtained by (Ashrafuzzaman et al., 2009) who reported that, inoculation with the plant growth promoting rhizobacteria like *Azotobcter*, had stimulation effect on the population of rhizosphere microorganisms and increased their numbers by more than 50% at the end of the experiment compared with the number recorded before planting.

Soil Enzymatic Activity

Soil enzymatic activity clearly affected with Salinity level, increasing salinity reduces enzymatic activity, while increasing humic acid concentration led to increased soil enzymatic activity. biofertilization treatment either single or mixed inoculation stimulate soil enzymes especially mixed inoculation. Dehydrogenase, Nitrogenase and phosphatase enzymes were measured to clarify the effect of the different used biofertilization treatments and humic acid concentrations on soil enzymatic activity. Soil enzymes varied within the different biofertilization treatments and HA levels. *Azotobacter chroococcum* inoculation as single treatment gave the higher values of soil enzymes than Mycorrhizae, while, mixed biofertilization

Table 9. Interaction effects of salinity, humic acid and biofertilization on macronutrients content at Olive rhizosphere at 2nd seasons.

Salinity ppm	Humic ml/L	Biofertilization treatments	1st season			2nd season		
			N ppm	P ppm	K ppm	N ppm	P ppm	K ppm
2000	0	Control	159	11.1	228	162	11.16	231
		A.chrococcum	182	12.15	236	186	12.24	239
		G.macrocarbium	160	14.23	243	161	14.36	245
		Mixture	193	14.94	251	197	15.21	255
	0.5	Control	159	11.12	231	162	11.19	255
		A.chrococcum	184	12.26	239	187	12.34	242
		G.macrocarbium	168	14.36	248	169	14.46	251
		Mixture	194	15.12	257	198	15.34	264
	1	Control	160	11.26	237	164	11.2	239
		A.chrococcum	192	12.71	246	198	12.42	249
		G.macrocarbium	169	14.4	261	171	14.52	268
		Mixture	196	15.3	278	203	15.43	286
	1.5	Control	163	11.3	241	165	11.21	244
		A.chrococcum	195	12.76	258	206	12.53	263
		G.macrocarbium	172	14.9	273	173	14.53	278
		Mixture	208	15.7	286	214	15.68	291
3000	0	Control	152	10.93	225	153	11.05	228
		A.chrococcum	179	11.69	234	184	11.75	238
		G.macrocarbium	158	13.9	239	162	14.2	242
		Mixture	189	14.12	248	193	14.24	251
	0.5	Control	155	11.15	228	158	11.19	231
		A.chrococcum	184	11.94	238	188	12.04	242
		G.macrocarbium	163	13.78	246	171	14.29	253
		Mixture	191	14.26	257	193	14.58	264
	1	Control	155	11.15	229	156	11.23	232
		A.chrococcum	188	11.98	241	192	12.2	243
		G.macrocarbium	165	13.85	253	171	14.36	258
		Mixture	198	14.61	260	202	14.82	271
	1.5	Control	158	11.16	230	160	11.29	234
		A.chrococcum	188	12.1	249	191	12.36	256
		G.macrocarbium	165	14.26	268	167	14.49	258
		Mixture	197	14.69	284	205	14.95	296
4000	0	Control	149	10.82	219	149	10.96	223
		A.chrococcum	178	11.45	227	182	11.58	231
		G.macrocarbium	156	13.28	235	158	13.45	239
		Mixture	185	13.71	239	189	13.96	243
	0.5	Control	153	11	220	155	11.06	225
		A.chrococcum	183	11.65	231	187	11.82	236
		G.macrocarbium	158	13.6	236	162	13.75	239
		Mixture	188	13.94	245	195	14.11	248
	1	Control	154	11.12	220	156	11.15	224
		A.chrococcum	184	11.72	236	189	11.86	237
		G.macrocarbium	161	13.46	243	165	13.89	248
		Mixture	193	14.1	249	197	14.36	251
	1.5	Control	154	11.25	221	155	11.34	223
		A.chrococcum	185	11.96	239	186	12.08	245
		G.macrocarbium	162	13.78	248	165	13.97	261
		Mixture	194	14.26	252	201	14.52	253
L.S.,D,at 5%			0.6509	0.1627	0.5971	0.4613	0.0187	0.6236

Table 10. Main effects of salinity, humic acid and biofertilization on Microbial determinations in Olive rhizosphere at two growing seasons.

Treatment	1st season				2nd season			
	Tc	Az	M%	Spore no.	Tc	Az	M%	Spoe
Salinity (ppm)								
2000	131.19	79.5	15.66	11.66	137.08	80.68	13.82	11.88
3000	92.88	585	13.3	10.96	97.92	59.77	13.9	11.43
4000	81.25	46.81	13.26	10.04	89.19	4958	16.7	10.5
L.S.D at 5%	0.209945	0.1680	0.0307	0.0162	0.1382	0.1478	0.0441	0.0261
Humic (ml/L)								
0	93.67	55.08	13	10.43	97.94	57.92	13.68	10.8
0.5	97.92	60.58	13.8	10.72	104.17	62.4	14.52	10.85
1.0	103.67	62.58	14.5	11.08	111.17	65.8	15.19	11.4
1.5	111.83	65.5	14.97	11.33	118.97	67.33	15.75	12.04
L.S.D at 5%	0.26284	0.2357	0.0299	0.0208	0.2069	0.1099	0.0453	0.0218
Biofertilization treatments								
Control	70.42	41.83	6.4	8.6	72.83	45.64	6.85	8.6
A.chrococcum	97.42	51	7.43	9.06	103.44	53.11	7.91	10.13
G.macrocarbium	109.75	71.5	20.56	12.77	117.11	72.44	21.34	12.9
Mixture	129.5	78.92	21.92	13.15	138.86	82.19	22.98	13.21
L.S.D at 5%	0.1242	0.2477	0.0280	0.0142	0.1021	0.1620	0.0451	0.0256

Table 11. Interaction effect of salinity, humic acid and biofertilization on Microbial determinations in Olive rhizosphere at two growing seasons.

Salinity ppm	Humic ml/L	Biofertilization treatments	1st season				2nd season			
			Tc	Az	M%	M spore	Tc	Az	M%	M spore
2000	0	Control	74	47	6.8	8.9	76	53	7.2	9.3
		A.chrococcum	118	83	8.2	9.2	122	85	8.8	9.8
		G.macrocarbium	132	62	20.8	12.8	139	63	21.3	13.3
		Mixture	159	94	22.1	13.2	164	97	22.9	13.7
	0.5	Control	77	52	7.5	9.1	79	53	7.9	9.4
		A.chrococcum	123	93	8.9	9.3	130	88	9.5	9.9
		G.macrocarbium	141	68	21.6	13.4	148	65	23.1	13.7
		Mixture	170	106	24.1	13.7	173	110	25.6	13.8
	1	Control	78	48	7.6	9.3	85	58	8.1	9.5
		A.chrococcum	129	97	9.2	9.7	133	93	9.6	9.8
		G.macrocarbium	148	70	22.4	14.2	156	69	23.9	14.4
		Mixture	184	112	24.8	14.8	179	114	27.1	14.9
	1.5	Control	86	50	7.9	9.5	89	59	8.6	9.6
		A.chrococcum	136	102	9.8	9.8	147	98	10.3	10.1
		G.macrocarbium	152	71	23.6	14.7	162	71	24.9	14.9
		Mixture	190	118	25.2	14.9	211	115	27.6	15.6
3000	0	Control	63	38	5.7	8.3	66	42	5.9	8.6
		A.chrococcum	76	60	6.2	8.9	81	61	6.7	9.2
		G.macrocarbium	85	44	18.9	12.5	89	47	19.3	13.1
		Mixture	104	63	19.3	12.9	113	69	19.8	13.4
	0.5	Control	68	43	5.7	8.7	71	44	6.2	8.8
		A.chrococcum	78	64	6.4	9.1	86	67	6.8	9.3
		G.macrocarbium	92	49	19.5	12.8	98	50	20.1	13.5
		Mixture	106	71	20.7	13.1	113	78	21.2	13.9
	1	Control	69	42	5.9	8.8	72	45	6.5	9.2
		A.chrococcum	95	69	6.7	9.3	103	73	7.3	9.8
		G.macrocarbium	98	47	20.3	12.9	114	52	20.6	13.7
		Mixture	113	74	21.8	13.2	121	79	22.4	14.2
	1.5	Control	70	46	6.1	8.9	71	46	6.9	9.1
		A.chrococcum	106	71	6.9	9.4	109	73	7.4	9.7
		G.macrocarbium	119	48	20.8	13.1	122	52	21.5	13.4
		Mixture	128	77	21.9	13.5	138	79	22.4	13.9

Table 11. Interaction effect of salinity, humic acid and biofertilization on Microbial determinations in Olive rhizosphere at two growing seasons.

Salinity ppm	Humic ml/L	Biofertilization treatments	1st season				2nd season			
			Tc	Az	M%	M spore	Tc	Az	M%	M spore
4000	0	Control	59	33	5.3	7.5	64	34	5.7	7.6
		A.chrococcum	72	48	5.9	8.3	77	51	6.3	8.8
		G.macrocarbium	82	37	18.2	11.2	89	38	19.7	11.5
		Mixture	93	52	18.7	11.4	95	56	20.5	11.9
	0.5	Control	61	33	5.8	7.7	63	37	6.1	7.8
		A.chrococcum	75	54	6.4	8.4	78	55	6.9	9.1
		G.macrocarbium	87	39	19.2	11.5	94	43	19.8	12.1
		Mixture	97	57	20.1	11.9	109	59	20.8	12.7
	1	Control	61	35	6	7.9	67	38	6.3	8.3
		A.chrococcum	77	58	7.1	8.5	81	62	7.5	8.8
		G.macrocarbium	88	40	20.6	11.9	94	43	20.9	12.6
		Mixture	102	59	21.4	12.4	117	63	21.8	13.2
	1.5	Control	65	36	6.2	8.1	69	38	6.5	8.4
		A.chrococcum	83	61	7.5	8.8	88	64	7.6	9.1
		G.macrocarbium	91	43	20.9	12.3	97	45	21.5	12.8
		Mixture	107	64	22.9	12.8	125	69	23.6	13.4
L.S.,D,at 5%			0.83.75	0.6719	0.1229	0.065	0.553	0.5912	0.1765	0.1044

TC: Total microbial counts $\times 10^5$cfu/g soil, Az:Azotobacter densities$\times 10^3$cells/g soil,M%: Mycorrhizal infection %, M Spore : Number of mycorrhizal spores

Table 12. Main effects of salinity, humic acid and biofertilization on microbial enzymes activities in rhizosphere of Olive in the two growing seasons.

treatment	1st season			2nd season		
	Dehydrogenase µlDHA/g dry soil	Nitrogenase µMC2H4kg/h	Phosphatase mg phenol/g soil/24h	Dehydrogenase µlDHA/g dry soil	Nitrogenase µMC2H4kg/h	Phosphatase mg phenol/g soil/24h
Salinity(ppm)						
2000	1.99	0.37	0.17	2.03	0.39	0.18
3000	1.84	0.32	0.15	1.89	0.38	0.16
4000	1.76	0.27	0.14	1.82	0.29	0.14
L.S.D at 5%	0.0186	0.47	0.07	0.832	0.0107	0.0107
Humic (ml/L)						
0	1.79	0.27	0.14	1.83	0.32	0.15
0.5	1.85	0.29	0.15	1.91	0.34	0.16
1.0	1.88	0.33	0.16	1.92	0.37	0.17
1.5	1.93	0.36	0.17	1.98	0.402	0.18
L.S.D at 5%	0.0198	0.077	0.0102	0.104	0.171	0.114
Biofertilization treatments						
Control	1.74	0.22	0.097	1.77	0.25	0.106
A.chrococcum	1.82	0.35	0.14	1.88	0.4	0.151
G.macrocarbium	1.93	0.3	0.17	1.97	0.33	0.183
Mixture	1.97	0.39	0.2	2.02	0.45	0.21
L.S.D at 5%	0.0153	0.013	0.018	0.168	0.0178	0.0137

Table 13. Interaction effects of salinity, humic acid and biofertilization on microbial enzymes activities in rhizosphere of Olive in the two growing seasons.

Salinity ppm	Humic ml/L	Biofertilization treatments	1st season			2nd season		
			Dehydrogenase µlDHA/g dry soil	Nitrogenase µMC2H4kg/h	Phosphatase mg phenol/g soil/24h	Dehydrogenase µlDHA/g dry soil	Nitrogenase µMC2H4kg/h	Phosphatase mg phenol/g soil/24h
2000	0	Control	1.78	0.22	0.096	1.81	0.25	0.11
		A.chrococcum	1.82	0.37	0.14	1.88	0.41	0.15
		G.macrocarbium	1.89	0.31	0.18	1.9	0.33	0.19
		Mixture	1.94	0.39	0.19	1.95	0.45	0.21
	0.5	Control	1.83	0.25	0.1	1.84	0.28	0.12
		A.chrococcum	1.96	0.38	0.16	1.98	0.46	0.16
		G.macrocarbium	2.02	0.34	0.18	2.06	0.36	0.2
		Mixture	2.14	0.41	0.21	2.15	0.47	0.22
	1	Control	1.89	0.27	0.11	1.9	0.28	0.13
		A.chrococcum	2.01	0.48	0.17	2.05	0.48	0.18
		G.macrocarbium	2.12	0.39	0.19	2.16	0.39	0.21
		Mixture	2.18	0.44	0.22	2.21	0.48	0.23
	1.5	Control	1.9	0.27	0.11	1.92	0.3	0.13
		A.chrococcum	2.08	0.51	0.19	2.15	0.53	0.18
		G.macrocarbium	2.19	0.43	0.2	2.24	0.44	0.22
		Mixture	2.25	0.49	0.23	2.29	0.56	0.24
3000	0	Control	1.66	0.21	0.09	1.69	0.23	0.093
		A.chrococcum	1.78	0.28	0.13	1.82	0.38	0.15
		G.macrocarbium	1.86	0.23	0.15	1.88	0.31	0.16
		Mixture	1.88	0.34	0.17	1.93	0.42	0.19
	0.5	Control	1.7	0.22	0.095	1.71	0.26	0.1
		A.chrococcum	1.74	0.31	0.14	1.96	0.42	0.15
		G.macrocarbium	1.91	0.28	0.17	1.98	0.36	0.18
		Mixture	1.93	0.39	0.19	2.05	0.45	0.2
	1	Control	1.72	0.22	0.1	1.73	0.26	0.11
		A.chrococcum	1.75	0.34	0.15	1.78	0.44	0.16
		G.macrocarbium	1.93	0.31	0.18	1.99	0.37	0.19
		Mixture	1.98	0.45	0.2	2.03	0.48	0.21
	1.5	Control	1.77	0.24	0.1	1.78	0.28	0.11
		A.chrococcum	1.82	0.39	0.16	1.89	0.47	0.18
		G.macrocarbium	1.97	0.35	0.19	1.98	0.4	0.19
		Mixture	2.03	0.48	0.21	2.11	0.54	0.22
4000	0	Control	1.63	0.18	0.082	1.68	0.21	0.086
		A.chrococcum	1.72	0.25	0.11	1.75	0.27	0.12
		G.macrocarbium	1.79	0.21	0.14	1.86	0.22	0.15
		Mixture	1.8	0.29	0.17	1.82	0.31	0.19
	0.5	Control	1.64	0.18	0.088	1.73	0.2	0.09
		A.chrococcum	1.72	0.27	0.11	1.79	0.29	0.12
		G.macrocarbium	1.8	0.23	0.15	1.86	0.25	0.16
		Mixture	1.82	0.33	0.19	1.82	0.34	0.2
	1	Control	1.66	0.19	0.09	1.69	0.2	0.094
		A.chrococcum	1.73	0.28	0.12	1.78	0.31	0.13
		G.macrocarbium	1.81	0.27	0.15	1.88	0.28	0.16
		Mixture	1.84	0.36	0.19	1.89	0.42	0.2
	1.5	Control	1.69	0.2	0.096	1.76	0.21	0.1
		A.chrococcum	1.75	0.34	0.13	1.78	0.35	0.15
		G.macrocarbium	1.87	0.29	0.17	1.94	0.3	0.19
		Mixture	1.89	0.39	0.2	2.01	0.44	0.21
L.S.,D,at 5%			0.0371	0.0186	0.0191	0.0177	0.0206	0.0260

treatment surpassed all individual treatments. Many investigators demonstrated the positive effect of dual inoculation with N_2-fixer and P-solubilizes on N_2-ase activity (El- Komy, 2005).

Also, the addition of biofertilization treatments and HA level had a pronounced positive action on the quality of nitrogenase and phosphatase enzymes in both growing seasons. All biofertilization treatments improved the microbial activity in the rhizosphere zone and recorded significant increases, compared to the un-inoculated treatment. These increases may be due to production of phytohormones such as Indoleacetic acid (IAA),gibberellic acid, cytokinins and ethylene (Glick, 1995), a symbiotic N_2 fixation (Dobbelaera *et al.*, 2003), antagonism against phytopathogenic microorganisms by production of siderophores (Scher and Baker, 1982), solubilization of mineral phosphates and other nutrients (De Freitas *et al*., 1997).

CONCLUSION

Biofertilization and humic acid application have the potential to improve Olive (Picual) seedling growth under salinity stress. Mixed biofertilization treatments showed synergistic effect toward elevate salinity stress on Olive seedlings and improve growth parameters. Humic acid application has a beneficial effect not only to plant growth and soil but to biofertilizers applied as well. Finally from this study we can concluded that Olive (Picual) seedling achieved the highest values in all studied parameter under salinity 2000 ppm plus humic acid 1.5 % with, Azotobacter *chroococcum* and Myccorhizae as mixed biofertilization treatment.

REFERENCES

Ashrafuzzaman, M., A. H. R. I. M.Farid, H. M. d.Anamul, I. S. M.Zahurul, Shahidullah, and S. Meon. 2009. Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice S. M. growth. African J. of Biotechnol, 8 (7): 1247-1252.

Augé, R.M. 2001.Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42.doi:10.1007/s005720100097

Becking, J.H. 2006. The Family Azotobacteraceae. In: Dworkin M., Schleifer Falkow S., Rosenberg E., Schleifer K.-H. and Stackebrandt E. The Prokaryotes, Vol. 6, 3rd edition. Singapore: Springer Science + Business Media.759 - 78.

Berg, G. 2009. Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol 84:11–18. doi:10.1007/s00253-009-20 92–7

Canbolat, M., S. Bilen, R. Çakmakçı, F. Şahin and A. Aydın. 2006. Effect of plant growth-promoting bacteria and soil compaction on barley seedling growth, nutrient uptake, soil properties and rhizosphere microflora. Biol Fertil Soils 42:350–357. doi:10.1007/s00374-005-0034-9

Chen, Y., M. De Nobili and T. Aviad, 2004. Stimulatory Effects of Humic Substances on Plant

David, P.P., P.V. Nelson and D.C. Sanders.1994.A humic acid improves growth of tomato seedling in solution culture. J. plant Nutr. 17: 173-184.

De Freitas J. R., M. R. Banerjee and J. J. Germida. 1997. Phosphate solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (Brassica napus L.). Biol. Fertil. Soils. 24: 358-364.

Dobbelaere, S., J. Vanderleyden and Y.Okon. 2003.Plant growth-promoting effects of diazotrophs in the rhizosphere. CRC Crit Rev Plant Sci.;22:107–149.

Dodd, I.C. and J.M. Ruiz-Lozano 2012. Microbial enhancement of crop resource use efficiency. Curr Opin Biotechnol 23:236–242.doi:10.1016/j.copbio.2011.09.005.

Eissa, F.M., M.A. Faith and S.A. El-Shall. 2007. The role of humic acid and rootstock in enhancing salt tolerance of " Le-Conte" pear seedlings.J.Agric. Sci. Mansoura Univ. 32(5): 3651-3666.

El-Komy, H.M.A. 2005. Coimmobilization of *Azospirillum lipoferum* and *Bacillus megatherium* for successful phosphorus and nitrogen nutrition of wheat plants. Food Technol. Biotechnol., 43(1):19-27.

El-Wakeil, N. E., and T. N. El-Sebai. 2007. Role of biofertilizer on faba bean growth, yield, and its effect on bean aphid and the associated predators. Res. J. Agric. Biol. Sci, 3(6): 800-807.

Gamalero, E., M. G.Martinotti, A. Trotta, P. Lemanceau, and G. Berta. 2002.Morphogenetic modifications induced by Pseudomonas fluorescens A6RI and Glomus mosseae BEG12 in the root system of tomato differ according to plant growth conditions. New phytologist, 155(2): 293-300.

Gerdemann, J.W. and T.H. Nicolson .1963. Spores of mycorrhizal Endogone extracted from soil by wet sieving and decanting. Trans. Brit. Mycol. Soc. 46: 235-244.

Ghaith, M. W. 2009. Development of irrigation system on Manzanillo Olive trees under El-Maghara area conditions. Ph.D. thesis. Faculty of Environmental Agricultural Sciences (El-Arish), Suez Canal University.

Glick BR. 1995.The enhancement of plant growth by free-living bacteria. Can J Microbiol. ;41:109–117.

Giovannetti, M. and B. Mosse. 1980. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizae infection in roots, New Phytologist, 84: 489-500.

Hartwigsen, J. and R. Evansmichael. 2000. Humic acid and substrate treatments promote seedling root development. Hort. Sci. 7:1231-1233.

Leyval, C., K. Turnau and K. Haselwandter. 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. Mycorrhiza 7: 139–153

Magdi, T.A., E.M. Selim and A.M. El-Ghamry. 2011.Integrated effects of Bio and mineral fertilizers and humic substances on Growth, yield and nutrient contents of fertigated Cowpea (Vigna unguiculata L.) grown on sandy soils. Journal of Agron. 10(1): 34-39.

Massoud,.O.N., M.M.I. Afifi, Y.S. El-Akshar and G.A.M. El-Sayed.2013. Impact of biofertilizers and humic acid on the growth and yield of Wheat grown in reclaimed sandy soil. Res. J. of Agric. and Bio. Sci., 9(2): 104-113.

Muhammad, Y., A. Kaleem, M. Waqas and Asif T. 2012. Bio-fertilizers, substitution of synthetic fertilizers in cereals for leveraging agriculture. Crop and Environ. 3(1-2): 62-66.

Munns, R. and M. Tester. 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59: 651– 681.

Muscolo, A. and M. Sidari. 2007. Biological activity of humic substances is related to their chemical structure. Soil Sci. Soc. Am.J. 71: 75-85.

Nautiyal, C. S. 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiology Letters. 170: 265-270.

Page, A.L. , R.H. Miller and D.R. Keeney.1982.Methods of Soil Analysis. Parts 2. Am. Soc. Agro., Madison, W.1.

Pellerin, S., A. Mollier, C. Morel and C. Plenchette.2007. Effect of incorporation of Brassica napus L. residues in soils on mycorrhizal fungus colonization of roots and phosphorus uptake by maize (Zea mays L.). Europ. J. Agro. 26: 113 -120.

Querejeta, J.I, J.M. Barea, M.F.Allen, F. Caravaca, A. Roldan. 2003. Differential response of δ13C and water use efficiency to arbuscular mycorrhizal infection in two arid land woody plant species. Oecologia 135:510–515

Rojas-Tapias, D., A. Moreno-Galvan, S. Pardo-Diaz, M. Obando, D. Rivera and R. Bonilla. 2012. Effect of inoculation with plant growth-promoting bacteria (PGPB) on amelioration of saline stress in maize (Zeamays). Appl Soil Ecol 61:264–72. doi:10.1016/j.apsoil.2012.01.006

Scher, F. M., and R. Baker. 1982. Effect of *Pseudomonas putida* and a synthetic iron chelator on induction of soil suppressiveness to Fusarium wilt pathogens. Phytopathology. 72(12): 1567-1573.

Schmdit, W., S. Santi, R. Pinton and Z. Varanini .2007. Water- extractable humic substances alter root development and epidermal cell pattern in Arabidopsis. Plant &Soil 300: 259-267

Sepaskhah, A.R and N.Yarami. 2010. Evaluation of macroscopic water extraction model for salinity and water stress in saffron yield production. Inter. J. Plant Production. 4: 175- 186.

Snedecor, G. W., and W. G. Cochran. 1967.statistical methods, ed 6, Ames, Iowa, Iowa State University Press, Section, 12, 349-352.

Varanini, Z. and R. Pinton. 2001. Direct versus indirect effects of soil humic substances on plant growth and nutrition. In: The rhizosphere: biochemistry and organic substances at the soil-plant interface (Pinton R., Varanini Z., Nannipieri P., eds). Marcel Dekker Inc, NY, USA. pp. 141-157

Vessey, J.K. 2003. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255: 571–586. doi:10.1023/a:1026037216893

Vivas A, A. Marulanda, J. Ruiz-Lozano, J. Barea and R. Azcón. 2003. Influence of a Bacillus sp. on physiological activities of two arbuscular mycorrhizal fungi and on plant responses to PEGinduced drought stress. Mycorrhiza 13:249–256. doi:10.1007/s00572-003-0223-z

Waksman, S.A. and H.A. Lechevalier. 1962. Description of antibiotics. pp.206-307. In The Actinomycetes vol III: Antibiotics of Actinomycetes. The Williams & Wilkins Company, Baltimore.

Waller, R. A. and D. B. Duncan. 1969.A Bayes rule for the symmetric multiple comparisons problem. J. Am Statistical Association, 64(328):1484-1503.

Zandonadi, D.B., L.P.Canellas and A. Rocha Façanha. 2007. Indoleacetic and humic acids induce lateral root development through a concerted plasmalemma and tonoplast H+pumps activation. Planta, 225: 1583-1595.

Zhu, J.K., 2001. Plant salt tolerance. Trends Plant Sci., 6: 66-71.

الملخص العربي

تأثير بعض معاملات التسميد الحيوي والهيوميك اسيد على نمو شتلات الزيتون تحت ظروف مستويات مختلفة من الاجهاد الملحي

منى مرسى الشاذلي، وائل موسى غيث

تتواجد الاملاح طبيعيا فى الترب ومياه الري. الملوحه لها تأثير عكسي على نمو النبات والمجموع الخضري والجذري، تعمل على تقليل قدرة النبات على امتصاص الماء من التربة وتسبب انواع عديدة من ضعف النمو والانتاجيه

تم اجراء الدراسة الحالية لدراسة تأثير تركيزات من حمض الهيوميك (بدون – ٠,٥–١– ١,٥مل/لتر) وبعض معاملات التسميد الحيوى (كنترول– ازوتوباكتر كروكوككم – ميكروهيزا جلومس ماكروكاربيم) ومخلوط من الازوتوباكتر+ الميكروريزا على شتلات الزيتون صنف بيكوال النامية في محطة بحوث الشيخ زويد مركز بحوث الصحراء– محافظة شمال سيناء

وقد أظهرت النتائج طبقا لقياسات النمو ان معدل الملوحة (٢٠٠٠ جزء فى المليون) سجل أعلى قياسات معنوية لنمو شتلات الزيتون: الطول– قطر الجذع–عدد الافرع–عدد الاوراق–طول الورقة– عرض الورقة – مساحة سطح الورقة وايضا الوزن الخضرى والجاف للمجموع الخضرى والجذرى.

وكانت اقل قياسات تم تسجيلها لمستوى الملوحة (٤٠٠٠ جزء فى المليون خلال موسمى النمو) وقد اعطى معدل الملوحة ٢٠٠٠ جزء في المليون فرصة للنبات النامي لاستكمال كل العمليات الفسيولوجية في زمن قياسي أفضل من التركيزات العالية لمياه الري

وقد أدى التركيز العالي للملوحة الى حدوث نقص فى جميع القياسات المدروسة خلال موسمي النمو.

كما ان زيادة تركيز حمض الهيوميك من (٠,٥ الى ١,٥مل/لتر) أدى الى حدوث زيادة معنوية في جميع الصفات المدروسة مقارنة بالكنترول خلال موسمي النمو كما ادى تطبيق معاملات التسميد الحيوي سواء منفردة أو مخلوطة الى زيادة في معدلات نمو الشتلات تحت التركيزات المختلفة للملوحة

وأوضحت الدراسة ان استخدام مخلوط من الاسمدة الحيوية كان له تأثير معنوي على خصائص نمو شتلات الزيتون ووزن مجموعها الجذري والخضري ومحتوى اوراقها من العناصر الغذائية مقارنة بالكنترول والمعاملات الفردية

والتي تأثرت تأثرا معنويا ايجابيا بزيادة تركيز الهيوميك ومعاملات التسميد الحيوي المفردة والمجمعة

كما ان المعاملة بمخلوط من الاسمدة الحيوية ادت الى قيم عالية من الخواص الميكروبيولوجية للتربة مثل الاعداد الكلية للميكروبات– اعداد الازوتوباكتر–نسبة الاصابة بالميكروريزا– عدد جراثيم الميكروريزا /جم تربه– الانزيمات الميكروبية في التربة مثل الديهيدروجينيز– النيتروجينيز والفوسفاتيز ولذلك فانه لتخفيف الاثر السلبي للملوحة المرتفعة على شتلات الزيتون نوصى باستخدام حمض الهيوميك بتركيز ١,٥مل/لتر مع مخلوط من الازوتوباكتر والميكروريزا.