



## Possibility of Overcoming Salinity Disadvantages on Lettuce Plants Using Humic Acid and Mycorrhiza

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**ABSTRACT:** Two pot experiments were carried out during the two successive winter seasons of 2019 and 2020, in a private farm at Ahmed Rami village, El-Bostan area, EL- Beheira Governorate, Egypt. The main purpose of this work is to investigate the effect of humic acid and inoculation with mycorrhiza under different salinity levels on the growth and chemical properties of Crisp head lettuce, iceberg, (*Lactuca sativa* var. *capitata* L. 'Calmar'). The goal is to expand lettuce cultivation in areas irrigated with high salinity concentration water. This experiment included 24 treatments which were the combinations between four salinity levels (tap water, 2.0, 4.0 and 6.0 mS/cm), and six treatments; i.e., five ameliorative treatments (mycorrhiza), (humic acid at 1.5 g / L), (humic acid at 3.0 g / L), (humic acid at 1.5 g / L + mycorrhiza), (humic acid at 3.0 g / L + mycorrhiza) in addition to the treatment of distilled water as a control treatment. The experimental layout was a split-plot system in a randomized complete blocks design, whereas the salinity levels were arranged in the main plots and the soil application treatments of humic acid and mycorrhiza were randomly distributed in the sub-plots. The obtained results, generally, showed, that all values of the tested characters decreased with increasing salinity levels. The reduction rate on any character varied depending on the imposed level of salinity stress. Application of humic acid and /or mycorrhiza revealed significant effect in improving all studied characters as compared to the control treatment, in both seasons. Application of humic acid at 3.0 g / L + mycorrhiza achieved the highest average values of leaves number, head weight, total fresh weight, head dry weight, root length, root fresh weight, root dry weight, nitrogen, phosphorus, potassium, calcium, protein, total chlorophyll and potassium / sodium ratio and reduced sodium contents compare to the other treatments in both seasons. The combined treatment of humic acid at 3.0 g / L + mycorrhiza with tap water salinity level gave the highest mean values of the most tested characters. The conclusion of this research suggested the possibility of utilizing the combination between humic acid and mycorrhiza to enhance growth of lettuce plants and minimize the damaging effect of salinity.

**Keywords:** Lettuce; salinity; salt stress; humic acid; mycorrhiza; growth; chemical contents.

### INTRODUCTION

Edible crisped lettuce (*Lactuca sativa* var. *capitata* L. 'Calmar') belongs to the family *Asteraceae*, or *Compositae*. Lettuce was cultivated by the ancient Egyptians, Greeks and Romans and were widely spread to every continent and is grown everywhere except in the hottest tropical lowlands (Shannon and Grieve, 1999). The harvested area all over the world is 472552 fed. and the world production is 45449152 tones (FAOSTAT, 2019). In Egypt, production of lettuce is 203510 tones and the total area grown was 9592.8 fed. for lettuce and chicory (FAOSTAT, 2019). Lettuce is one of the most commonly consumed vegetables worldwide, but its nutritional value has been underestimated. Lettuce is low in sodium, fat and calories. It is a good source of iron, folate, vitamin C and fiber. Lettuce is also a good source of various other health-beneficial bioactive compounds (Kim *et al.*, 2016). However, lettuce is a salt moderately-

sensitive crop where salinity affects its quality, yield, and production (Grieve *et al.*, 2012).

Soil salinity is one of the major abiotic stresses that hamper crop growth and productivity worldwide. It has been stated that approximately 20% of irrigated land worldwide is salt-affected, which represents one-third of food-producing land (Gregory *et al.*, 2018). Moreover, the salt-affected areas are increasing at a rate of 10% yearly for various reasons, including low precipitation, high surface evaporation, poor cultural practices, and irrigation using saline water (Shrivastava and Kumar, 2015). This issue has been further aggravated by the continued trends in global warming and climatic changes. Therefore, living with salinity is the only way of supportive agricultural production in the salt affected soil. So that, it is must to finding the best management to alleviate salt hazard (Al-Rawahy *et al.* 2011).

In current years, exogenous protectants such as osmoprotectants, phytohormones, humic compounds, antioxidants and mycorrhiza have been found useful to alleviate the salt-induced damages (Khan *et al.*, 2019). The development of methods and strategies to ameliorate the harmful effects of salt stress on plants has received considerable attention (Senaratna *et al.*, 2000).

Humic acids are rich in mineral nutrients like potassium, calcium, magnesium, zinc, iron, copper and organic acid (Tahir *et al.*, 2011; Canellas *et al.*, 2015). Photosynthetic activity of lettuce improved with all levels of humic acid due to enhancement of chlorophyll content and mesophyll conductance. Humic acid can stimulate N metabolism and photosynthesis activity of lettuce to improve yield (Haghighi *et al.*, 2012). Aydin *et al.* (2012) indicated that adding Humic acid treatment to bean plants has great potential in alleviating salinity stress on plant growth in saline soils of arid and semi-arid areas and appeared to be highly effective for soil conditioners in vegetable growth, to improve crop tolerance and growth saline conditions and enhanced plant root and shoot dry weight by allowing nutrients and water to be released to the plant as needed. Also, El-Hamdi *et al.* (2016) indicated that humic acid exhibited a protective effect against salinity stress that increased fresh and dry weight and improving physicochemical and biological properties of saline soils.

Arbuscular Mycorrhizal Fungi -inoculated plants develop better than non-inoculated plants under salt stress, according to several studies. (Al-Karaki, 2000; Cantrell and Linderman, 2001; Giri *et al.*, 2003; Sannazzaro *et al.*, 2007; Zuccarini and Okurowska, 2008). Arbuscular Mycorrhizal Fungi are soil-borne fungi that can increase resistance to several abiotic stress factors and significantly improve plant nutrient uptake (Sun *et al.*, 2018). Arbuscular Mycorrhizal Fungi have the capability to improvement the uptake of inorganic nutrients in almost all plants, specifically of phosphate (Smith *et al.*, 2003; Nell *et al.*, 2010).

The present study therefore, was conducted to evaluate the potential of humic acid and mycorrhiza, to alleviate the salt-induced deleterious effects on growth and chemical characteristics of lettuce under the environmental conditions of El-Behera Governorate.

## MATERIALS AND METHODS

Two pots experiments were conducted at Ahmed Rami village, El-Bostan, EL- Beheira Governorate, Egypt, during the successive winter seasons of 2018 and 2019 to investigate the effect of humic acid and inoculation with vesicular-arbuscular mycorrhizal (VAM) fungi under different salinity levels on the growth and chemical properties of Crisp head lettuce, iceberg, (*Lactuca sativa* var. *capitata* L. 'Calmar'). The physical and chemical analyses of the soil (Table 1) were carried out before transplanting according to Black (1965).

**Table (1):** Some Physical and chemical properties of the experimental soil.

Seasons		2019	2020
Physical properties	Sand (%)	72.0	73.2
	Silt (%)	11.6	10.7
	Clay (%)	16.4	16.1
	Texture	lomay sand	lomay sand
Chemical properties	pH	8.49	8.45
	EC (dSm <sup>-1</sup> )	311.592	242
	Organic matter (%)	0.106	0.09
	Available N (ppm)	69.4167	70
	Available P (ppm)	6.29167	5.5
	Available K (ppm)	120.217	94.3
	Mg	7.4	7.2
	Ca	14.6667	11.32
	Na	56.8	49.3
	Cl	89.03	76
	HCO <sub>3</sub>	73.76	67
SO <sub>4</sub>	35.83	24.5	

The lettuce, iceberg seeds, cv. Calmar produced by Nelson Garden seed company based in Sweden, were purchased from a local seeds market, and sown in plastic pots (35 cm inner diameter, and 30 cm height), each pot was filled with 12 kg of soil, and placed in the open field. The seeds were planted on 10 and 13 of October 2019 and 2020, respectively. Each treatment was composed of five

replicated pots with four plants in each pot. Each experiment includes 24 treatments which were the combinations between four salinity levels (Tap water, 2.0, 4.0 and 6.0 mS/cm) and six treatments; i.e., five protecting treatments: humic acid at 1.5 g / L, humic acid at 3.0 g / L, inoculation of mycorrhizal (VAM), (humic acid at 1.5 g / L + mycorrhiza), (humic acid at 3.0 g / L + mycorrhiza)

in addition to the control treatment (distilled water). The source of humic acid is potassium humate.

**Mycorrhizal (VAM) root colonization:** the process of root infection takes place at the age of 20 days from planting. All VAM fungal structures (hyphae, arbuscules, and vesicles) found in the roots were counted. Stained root pieces were examined under a dissecting scope at 40X magnification and extent of colonization was assessed by the grid-line intercept method (Giovannetti and Mosse, 1980). The recommended concentrations of adding treatments were applied as a soil application. All precautions were followed during weighing, dissolving and adding. Each treatment was applied three times after planting. The first application was conducted in the three specific leaves phase (20 days) after sowing and the others were applied with one-week intervals (Smoleń and Sady 2012; Fouda, 2016). Harvesting was done after 50 days of planting during both seasons. All experimental pots received identical levels of nitrogen, phosphorus and potassium fertilizers. Ammonium nitrate (33.5% N) at the rate of 60 kg N/fed. was equally divided and side dressed after 21, 28 and 35 days after planting, Calcium super phosphate (15.5 % P<sub>2</sub>O<sub>5</sub>) at the rate of 150 kg P<sub>2</sub>O<sub>5</sub> /fed. was base dressed before planting and potassium sulphate (48 % K<sub>2</sub>O) at the rate of 50 kg K<sub>2</sub>O /fed. was equally divided and side dressed after 21 and 28 days of planting. All other agricultural practices were adopted whenever they were necessary and as commonly recommended for the commercial production of lettuce, iceberg.

The layout experiment was split plots system in a Randomized Complete Blocks Design (RCBD) with three replications. The salinity levels were arranged in the main plots and the protecting treatments of humic acid, mycorrhiza and their combination, were considered as sub- plots.

#### **Data Recorded**

##### **Vegetative growth parameters and chlorophyll contents**

Lettuce plants were harvested after 55 days from transplanting and the measurement of vegetative growth parameters were performed immediately. Three lettuce plants from each treatment were randomly taken to measure:

**Number of leaves per plant;** It was estimated as an average of the selected plants.

**Plant fresh weight (g);** The whole plant sample was weighted and the average weight plant<sup>-1</sup>(g) was calculated.

**Plant dry weight (g);** The collected plant samples were oven dried at 70 C° in a forced air oven till obtaining a constant weight to obtain shoots dry weigh (g plant<sup>-1</sup>) and the dried tissues were ground for further analysis., then the percentage of dry matter was calculated.

Total leaf chlorophyll contents (SPAD index); were measured using spad-502 chlorophyll meter devise (Konica Minolta, Kearney, NE, USA).

**Head weight (g);** was determined as the weight of the three selected randomly edible heads.

##### **Root characteristics**

**Root length (cm);** it was measured for plant samples randomly taken, and the average root length (cm) was calculated.

**Root fresh weight (g);** The whole fresh root sample was weighted and the average root weight (gm) was calculated

**Root dry weight (g);** The collected fresh root samples were oven dried at 70 C° in a forced air oven till obtaining a constant weight to obtain root dry weigh (g).

##### **Chemical contents**

After harvest, chemical contents were achieved immediately.

Total nitrogen was determined calorimetrically according to Evenhuis and De Waard (1980). Phosphorus was determined using ammonium molybdate stannous chloride method (A.O.A.C, 1992). Potassium and sodium were measured using a flame photometer as explained by Singh *et al.* (2005). Then the ratio of K/Na was calculated. Calcium was determined by using the versinate titration method, as described by Johnson and Ulrich (1959).

##### **Statistical analysis**

All the obtained data were statistically analyzed by CoStat program (Version 6.4, Co Hort, USA, 1998–2008). Least significant difference (LSD) test was applied at 0.05 level of probability to compare means of different treatments according to Williams and Abdi (2010).

## **RESULTS AND DISCUSSION**

The results of the two studied factors and their interaction on the several characters of lettuce plants during 2019 and 2020 seasons can be presented below three titles as follow: 1- Vegetative growth characters and root characteristics. 2- Chemical contents. 3- Chlorophyll and protein contents.

### **Mean performances of vegetative growth characters and root characteristics of lettuce**

Data presented in Tables (2 and 3) indicated that all values of the tested parameters decreased as salinity levels increased. The highest values of the given parameters were obtained from control treatment, whereas that of 6.0 mS/cm salinity gave the lowest ones, in both seasons. At salinity of 6.0 mS/cm, the estimated percentage reductions, expressed as leaves number, head weight, total fresh weight, head dry weight, Plant dry weight, root length, root fresh weight and root dry weight, were (51.76 and 51.24 %), (59.12 and 59.41 %), (61.38 and 61.61 %), (60.36 and 60.62%), (61.11

and 61.15 %), (46.63 and 46.28 %), (80.66 and 80.62%) and (64.60 and 63.66%) compared to the control treatment in the first and second season, respectively. The adverse effects of high salinity on plants are connected to the subsequent factors: (1) low water potential of soil solution (water stress), (2) nutritional imbalance and disturbing ionic homeostasis (ionic stress), (3) specific ion effect (salt stress), (4) over-production of reactive oxygen species (oxidative stress) (Parvaiz and Satyawati, 2008; Hasanuzzaman *et al.*, 2013). Salinity stress is known to retard plant growth through its effect on several dynamic factors of plant metabolism, including osmotic adjustment (Sakr and El-Metwally, 2009) nutrient uptake, protein and nucleic acid synthesis, photosynthesis (Zaibunnisa, *et al.*, 2002), organic solute accumulation, enzyme activity, hormonal balance and reduced water availability at the cell level and then reduced plant growth and finally reduced yield. Meanwhile, the effect of soil salinity on lettuce yield was significant. Maximum yield was obtained from the control treatment, and lettuce yield decreased as soil salinity increased (Ünlükara *et al.*, 2008, and Silva *et al.*, 2019). Also, in spinach, the tested characters of plant height, plant fresh weight, plant dry weight, number of leaves per plant, root length, root fresh weight and root dry weight decreased with increasing salinity levels. The reduction rate on any character varied depending on the imposed level of salinity stress (Gabr *et al.*, 2022). Furthermore, in tomato, increasing salinity was accompanied by significant reductions in shoot weight, root length, and root surface area per plant (Mohammad *et al.*, 1998). In spinach, the fresh shoot and dry matter of spinach was affected negatively by salinity (Sheikhi and Ronaghi., 2012; and Ünlükara *et al.*, 2017). Likewise, Kaya *et al.* (2001) found that salinity significantly decreased spinach shoots fresh weight and dry weight, leaf relative water content, and specific leaf area compared to control treatment. The growth reduction was produced by the osmotic effect of salt outside the roots, and following growth reduction was caused by the inability to prevent salt from reaching toxic levels in transpiring leaves (Hniličková, *et al.*, 2019). Concerning the main effect of the protection treatments (humic acid and mycorrhiza) on the leaves number, head weight, total fresh weight, head dry weight, Plant dry weight, root length, root fresh weight and root dry weight of lettuce plants, results existing in Tables (2 and 3) revealed that addition of humic acid and mycorrhiza showed significant effect in most studied characters, except root dry weight on humic acid at 1.5 g in the first season only, compared to the control treatment, in both seasons. For example, application of humic acid at 3.0 g / L + mycorrhiza recorded, generally, the highest average values of leaves number, head

weight, total fresh weight, head dry weight, Plant dry weight, root length, root fresh weight and root dry weight compared to the other treatments, in both seasons. However, the differences between the three treatments (mycorrhiza), (humic acid at 1.5 g/L + mycorrhiza) and (humic acid at 3.0 g / L + mycorrhiza) were not significant in head weight, total fresh weight and head dry weight in the first season, in addition leaves number and head dry weight in the second season. Moreover, the differences between humic acid at 3.0 g/L + mycorrhiza and both (humic acid at 1.5 g + mycorrhiza) and mycorrhiza in most cases were not significant. This specific treatment (humic acid at 3.0 g / L + mycorrhiza) the estimated percentages increase in leaves number, head weight, total fresh weight, head dry weight, Plant dry weight, root length, root fresh weight and root dry weight were (42.92+39.37 %), (22.81+22.27 %), (23.03+22.85 %), (25.57+25.67 %), (27.35 and 28.22%), (35.61 and 39.86 %), (25.57 and 29.62 %) and (36.56 and 42.05%) compared to the control treatment in the first and second season, respectively. The current results could be attributed to the role of each ameliorative material. In this respect, Humic acids are rich in mineral nutrients like potassium, calcium, magnesium, zinc, iron, copper and organic acids (Tahir *et al.*, 2011; Canellas *et al.*, 2015). Humic compounds have been shown to stimulate plant growth in terms of increasing plant height and dry or fresh weight as well as enhancing nutrient uptake (Malan, 2015). Humic substances can improve nutrient applications, increase chlorophyll production, improve seed germination, enhance fertilizers, and ultimately strengthening plants (Kandil *et al.*, 2020). Thus, the plants that grow in soils containing adequate amounts of humic acid are less stressed because humic substances are an important part of soil organic matter and shows anti-stress effects (Hanafy *et al.*, 2010). Humic acid application as well significantly increased the head weight of lettuce (Türkmen *et al.*, 2004). Sandepogu *et al.*, (2019) found that humic acid increased fresh and dry weight of lettuce and spinach plants. El-Hamdi *et al.* (2016) indicated that humic acid exhibited a protective effect against salinity stress. Applications of humic acid produced significant increases in shoot length, root length, number of leaves, fresh weights of stems and roots and dry weights of stem and root of pepper (*Capsicum annuum*) plants grown under salt stress as compared with untreated plants (Akladios and Mohamed 2018). The humic acid advanced [nutrient uptake](#), photosynthetic pigment concentrations and yield of chicory (Gholami *et al.*, 2019).

Mycorrhization, has been shown to improve the host plant's fitness by increasing its growth and biomass. Arbuscular Mycorrhizal Fungi -

inoculated plants develop better than non-inoculated plants under salt stress, according to several studies. (Al-Karaki, 2000; Cantrell and Linderman, 2001; Giri *et al.*, 2003; Sannazzaro *et al.*, 2007; Zuccarini and Okurowska, 2008). The large hyphae of the fungus allow them to explore greater soil volume than non-mycorrhizal plants, allowing them to raise P concentration in plants by enhancing its uptake (Ruiz-Lozano and Azco'n, 2000). Increased P uptake by AMF in saline-grown plants may reduce the negative effects of Na<sup>+</sup> and Cl<sup>-</sup> ions by maintaining vacuolar and selective ion intake (Rinaldelli and Mancuso, 1996), preventing ions from interfering with growth metabolic pathways (Cantrell and Lindermann, 2001). According to Al-Karaki (2000), a mycorrhizal tomato plant had higher shoot and root dry weight, fresh fruit yield, fruit weight, and fruit quantity than a non mycorrhizal tomato plants. When Cucurbita pepo plants colonized with Glomus intraradices were exposed to salinity stress, Colla *et al.* (2008) found better growth, yield, hydration status, nutrient content, and quality of fruits. Cantrell and Linderman (2001) reported that dry

shoot masses of VAM lettuce and onion plants were significantly greater than those of non-VAM plants. In addition, [Santander \*et al.\* \(2019\)](#) in lettuce, reported that the mycorrhizal plants had higher biomass production, increased synthesis of N uptake, and clear changes in ionic relations, particularly reduced accumulation of Na<sup>+</sup>, than those non-mycorrhizal plants under stress conditions.

Concerning the interaction effect between salinity levels and the ameliorative treatments (humic acid and mycorrhiza) on leaves number, head weight, total fresh weight, head dry weight, Plant dry weight root length, root fresh weight and root dry weight of lettuce plants, results presented in Tables (2 and 3) revealed significant differences among the means of their interactions between both variables. In general, the combination between zero salinity and humic acid at 3.0 g / L + mycorrhiza inoculation reached the highest average values of the above aforementioned characters in both seasons compared to other tested treatments.

**Table (2):** Mean values of lettuce vegetative growth characters as affected by salinity levels and soil application of humic acid and mycorrhiza and their interaction during winter seasons of 2019 and 2020.

Treatments	Leaves number		Head weight (g)		Total fresh weight (g)		Head dry weight (g)		Plant dry weight (g)		
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020	
<b>Salinity levels (mS/cm)</b>											
<b>Tap water</b>	29.17A*	30.06A	318.06A	319.56A	354.92A	357.00A	29.39A	29.54A	35.56A	35.95A	
<b>2.0</b>	26.33B	26.11B	285.00B	284.28B	309.77B	309.42B	22.80B	22.77B	27.23B	27.19B	
<b>4.0</b>	23.00C	23.06C	229.08C	225.87C	244.62C	241.19C	15.22C	15.01C	17.93C	17.68C	
<b>6.0</b>	14.22D	14.50D	129.09D	130.64D	136.24D	137.88D	11.57D	11.71D	13.81D	13.98D	
<b>Ameliorative treatments</b>											
<b>Control</b>	18.42E	18.25D	208.47C	208.87E	226.05C	227.13E	17.01C	17.13C	20.16D	20.44D	
<b>Humic 1 (H<sub>1</sub>)**</b>	21.58D	21.58C	235.25B	233.17D	255.70B	254.04D	19.29B	19.13B	22.70C	22.81C	
<b>Humic 2 (H<sub>2</sub>)</b>	23.42C	23.58B	240.58B	239.42C	261.80B	260.58C	19.51B	19.46B	23.33C	23.18C	
<b>Mycorrhiza (M)</b>	25.33AB	25.67A	249.22A	248.52B	271.60A	270.84B	20.67A	20.61A	24.93B	24.85B	
<b>H<sub>1</sub> + M</b>	24.67B	25.42A	253.43A	254.04A	275.48A	276.19A	20.64A	20.70A	24.83B	24.90B	
<b>H<sub>2</sub> + M</b>	25.67A	26.08A	254.90A	256.51A	277.69A	279.45A	21.37A	21.51A	25.85A	26.03A	
<b>Water salinity levels × ameliorative treatments interaction</b>											
<b>Salinity levels</b>	<b>Ameliorative treatments</b>										
<b>Tap water</b>	<b>Control</b>	23.67hij	18.25d	286.00d	293.33de	315.67c	326.21de	26.31c	27.06c	31.29d	32.60d
	<b>Humic 1</b>	25.00gh	21.58c	302.00bc	299.33cd	338.33d	335.38d	29.48b	29.22b	34.19c	35.10c
	<b>Humic 2</b>	29.67ed	23.58b	307.33b	309.33c	344.33d	346.58c	29.10b	29.29b	35.14c	35.03c
	<b>Mycorrhiza</b>	33.00a	25.67a	333.00a	330.67b	373.00a	370.42b	30.03ab	29.83ab	36.93b	36.68bc
	<b>H<sub>1</sub> + M</b>	31.00bc	25.42a	340.67a	343.33a	378.87a	381.82a	30.07ab	30.29ab	36.96b	37.23ab
	<b>H<sub>2</sub> + M</b>	32.67ab	26.08a	339.33a	341.33a	379.33a	381.57a	31.37a	31.55a	38.83a	39.07a
<b>2.0 (mS/cm)</b>	<b>Control</b>	21.67k	24.00f	266.33e	268.33g	288.20d	290.37h	19.74g	19.91g	23.77h	23.97g
	<b>Humic 1</b>	24.00hi	25.00ef	291.33cd	281.33f	314.77c	306.45g	21.54f	20.82fg	25.78g	24.91fg
	<b>Humic 2</b>	27.00ef	25.67e	283.33d	281.00f	308.47c	305.96g	22.41ef	22.26ef	26.84fg	26.66f
	<b>Mycorrhiza</b>	28.33de	30.67bc	287.67d	288.67ef	314.00c	315.09fg	24.21d	24.29d	28.85e	28.96e
	<b>H<sub>1</sub> + M</b>	28.33de	33.67a	288.00d	290.67def	313.92c	316.84ef	23.97de	24.20de	28.41ef	28.67e
	<b>H<sub>2</sub> + M</b>	28.67de	32.33ab	293.33cd	295.67de	319.27c	321.81ef	24.93cd	25.13cd	29.73de	29.97e
<b>4.0 (mS/cm)</b>	<b>Control</b>	17.00l	33.00a	186.67h	170.67k	199.80g	182.67l	13.73jki	12.60k	15.57jk	14.28l
	<b>Humic 1</b>	22.00jk	21.00h	229.67g	233.00ij	245.67f	249.24jk	14.45ij	14.69ij	17.03j	17.30ij
	<b>Humic 2</b>	23.00ijk	23.33fg	236.00fg	231.00j	252.27ef	246.94k	13.97jk	13.68jk	16.69j	16.34jk
	<b>Mycorrhiza</b>	25.00gh	26.67de	235.33fg	235.33hij	250.77ef	250.77ijk	15.97hi	15.97hi	18.94i	18.94hi
	<b>H<sub>1</sub> + M</b>	24.67ghi	28.33d	243.67f	242.00hi	259.77e	257.99ij	16.39h	16.28hi	19.41i	19.27hi
	<b>H<sub>2</sub> + M</b>	26.33fg	28.67cd	243.13fg	243.23h	259.43e	259.55i	16.82h	16.83h	19.93i	19.94h
<b>6.0 (mS/cm)</b>	<b>Control</b>	11.33n	28.67cd	94.87k	103.13n	100.53g	109.29p	8.24n	8.97l	10.01m	10.90m
	<b>Humic 1</b>	15.33lm	15.67i	118.00j	119.00m	124.03i	125.09o	11.70m	11.80k	13.80i	13.91l
	<b>Humic 2</b>	14.00m	22.33gh	135.67i	136.33l	142.13h	142.85n	12.54klm	12.60k	14.64kl	14.70kl
	<b>Mycorrhiza</b>	15.00m	22.00gh	140.87i	139.40l	148.63h	147.09mn	12.46klm	12.33k	14.99kl	14.84kl
	<b>H<sub>1</sub> + M</b>	14.67m	25.67e	141.37i	140.17l	149.37h	148.10mn	12.13lm	12.03k	14.53kl	14.41kl
	<b>H<sub>2</sub> + M</b>	15.00m	25.67e	143.80i	145.80l	152.73h	154.86m	12.37klm	12.54k	14.92kl	15.13kl

\*Means having the same alphabetical letter (s) in column, within a comparable group of means, do not significantly differ, using the revised L.S.D. test at  $p = 0.05$  level of probability.

\*\*H<sub>1</sub>=Humic acid at 1.5 g/L, H<sub>2</sub>= Humic acid at 3.0 g/L, M= Mycorrhiza.

**Table (3):** Mean values of lettuce root characteristics as affected by salinity levels and soil application of humic acid and mycorrhiza and their interaction during winter seasons of 2019 and 2020.

Treatments	Root length (cm)		Root fresh weight (g)		Root dry weight (g)		
	2019	2020	2019	2020	2019	2020	
<b>Salinity levels (mS/cm)</b>							
Tap water	21.73A*	22.11A	36.87A	37.44A	6.16A	6.41A	
2.0	18.00B	17.97B	25.20B	25.14B	4.43B	4.42B	
4.0	15.67C	15.48C	15.54C	15.32C	2.71C	2.67C	
6.0	11.67D	11.80D	7.14D	7.24D	2.24D	2.27D	
<b>Ameliorative treatments</b>							
Control	13.07E	13.57E	17.58C	18.27D	3.16C	3.31D	
Humic 1 (H <sub>1</sub> )**	16.55D	16.44D	21.10B	20.87C	3.41C	3.68C	
Humic 2 (H <sub>2</sub> )	17.16C	17.09C	21.22B	21.17BC	3.82B	3.73C	
Mycorrhiza (M)	17.62BC	17.57BC	22.38A	22.33AB	4.26A	4.25B	
H <sub>1</sub> + M	17.93AB	17.96AB	22.06AB	22.14A	4.19A	4.20B	
H <sub>2</sub> + M	18.28A	18.40A	22.79A	22.94A	4.48A	4.51A	
<b>Water salinity levels × ameliorative treatments interaction</b>							
Salinity levels	Ameliorative treatments						
Tap water	Control	19.67c	13.57e	29.67c	32.88d	4.98c	5.54c
	Humic 1	21.00b	16.44d	36.33b	36.05c	4.71cde	5.88c
	Humic 2	20.93b	17.09c	37.00b	37.24bc	6.04b	5.74c
	Mycorrhiza	22.67a	17.57Bc	40.00a	39.76a	6.90a	6.85b
	H <sub>1</sub> + M	22.97a	17.96ab	38.20ab	38.48ab	6.89a	6.94b
	H <sub>2</sub> + M	23.13a	18.40a	40.00a	40.24a	7.47a	7.51a
2.0 (mS/cm)	Control	14.67g	20.60 cd	21.87e	22.04f	4.03e	4.06e
	Humic 1	17.20de	21.86bc	26.03d	25.12e	4.24de	4.10e
	Humic 2	18.67c	20.84cd	25.13d	24.96e	4.44cde	4.40de
	Mycorrhiza	18.73c	21.07c	26.33d	26.43e	4.64cde	4.66d
	H <sub>1</sub> + M	19.07c	22.52ab	25.92d	26.17e	4.44cde	4.48de
	H <sub>2</sub> + M	19.67c	23.13a	25.93d	26.14e	4.80cd	4.84d
4.0 (mS/cm)	Control	10.93i	23.27a	13.13g	12.00h	1.84ij	1.69j
	Humic 1	15.67fg	14.78i	16.00f	16.24g	2.58fgh	2.62gh
	Humic 2	16.80de	16.60gh	16.27f	15.94g	2.72fgh	2.66gh
	Mycorrhiza	16.21ef	18.53f	15.43f	15.44g	2.97fg	2.97fg
	H <sub>1</sub> + M	17.17de	18.80ef	16.10f	15.99g	3.01fg	2.99fg
	H <sub>2</sub> + M	17.23d	19.25ef	16.30f	16.31g	3.11f	3.12f
6.0 (mS/cm)	Control	7.00j	19.83de	5.67k	6.16j	1.77j	1.93j
	Humic 1	12.33h	9.98k	6.03jk	6.09j	2.09hij	2.11ij
	Humic 2	12.23h	15.89hi	6.47ijk	6.52j	2.10hij	2.10ij
	Mycorrhiza	12.87h	16.47gh	7.77hij	7.69ij	2.53fghi	2.50hi
	H <sub>1</sub> + M	12.53h	16.21gh	8.00hi	7.93ij	2.40ghij	2.38hi
	H <sub>2</sub> + M	13.07h	17.05gh	8.93h	9.06i	2.55fgh	2.59gh

Means having the same alphabetical letter (s) in column, within a comparable group of means, do not significantly differ, using the revised L.S.D. test at  $p = 0.05$  level of probability.

\*\*H<sub>1</sub>=Humic acid at 1.5 g/L, H<sub>2</sub>= Humic acid at 3.0 g/L, M= Mycorrhiza.

### Mean performances of chemical contents of lettuce

Concerning the main effect of salinity levels on the percentages of nitrogen, phosphorus, potassium, calcium, sodium, potassium/sodium ratio, results are presented in Table (4) shown that most tested parameters decreased as salinity levels increased, except for sodium percentages which increased as salinity levels increased. The reduction rate on any character varied depending on the level of imposed salinity stress. The highest values of the given parameters (except sodium) were obtained from control treatment, while that of 6.0 mS/cm salinity gave the lowest ones, in both seasons. At salinity of 6.0 mS/cm, the estimated percentage reductions, expressed as nitrogen, phosphorus, potassium and calcium, were (24.35 and 19.33%), (40.00 and 36.21 %), (26.11 and 23.32%) and (24.22 and 24.26 %) for the first and second seasons, respectively and relative to the control treatment. The current results were in harmony with several investigators (Rogers *et al.* 2003; Hu and Schmidhalter 2005) who reported that nutritional disorders may result from the effect of salinity on nutrient availability, competitive uptake, transport, or distribution within the plant. Also, numerous reports indicated that salinity reduces nutrient uptake and accumulation of nutrients into the plants and it can differently affect the nutrient concentrations in plants depending upon crop species and salinity levels (Oertli, 1991). Salinity can decrease N accumulation in plants (Feigin *et al.*, 1991; Pessarakli, 1991 and Al-Rawahy *et al.*, 1992). Similarly, salinity could reduce nitrogen accumulation in plants. Decreased N uptake under saline situations occurs due to interaction between  $\text{Na}^+$  and  $\text{NH}_4^+$  and/or between  $\text{Cl}^-$  and  $\text{NO}_3^-$  that ultimately reduce the growth and yield of the crop. This reduction in  $\text{NO}_3^-$  uptake is connected with  $\text{Cl}^-$  antagonism or reduced water uptake under saline conditions (Lea-Cox and Syvertsen, 1993; Rozeff, 1995; Bar *et al.*, 1997). Examples of such an effect have been found in cucumber, (Martinez and Cerda, 1989), melon, (Feigin *et al.*, 1987), tomato (Martinez and Cerda, 1989). Spanish (Gabr *et al.* 2022). Many attributed this reduction to  $\text{Cl}^-$  antagonism of  $\text{NO}_3^-$  uptake (Bar *et al.*, 1997; Feigin *et al.*, 1987; Kafkafi *et al.*, 1982) while others attributed the response to salinity's effect on reduced water uptake (Lea-Cox and Syvertsen, 1993). High salinity can increase the uptake of  $\text{Na}^+$  and  $\text{Cl}^-$  from the soil, therefore suppressing the transport of other essential nutrients such as N, P, K, and Ca (Shrivastava and Kumar, 2015; Safdar *et al.*, 2019). Kim *et al.*, (2021) found that increasing NaCl concentration decreased amounts of minerals ( $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Fe}^{2+}$ ) in leaves of spinach and changed ratios of  $\text{Na}^+:\text{K}^+$  and  $\text{Na}^+:\text{Ca}^{2+}$ . Qadir and Schubert (2002) showed that availability of phosphorous is reduced in saline

soil due to (a) ionic strength effects that reduced the activity of  $\text{PO}_4^{3-}$ , (b) phosphate concentrations in soil solution was tightly controlled by sorption processes, and (c) low solubility of Ca-P minerals. Hence, it is noteworthy that phosphate concentration in agronomic crops decreases as salinity increases.

Under saline conditions, external  $\text{Na}^+$  not only interfere with  $\text{K}^+$  acquisition by the roots, but also may disrupt the integrity of root membranes and alter their selectivity. The selectivity of the root system for  $\text{K}^+$  over  $\text{Na}^+$  must be sufficient to meet the levels of  $\text{K}^+$  required for metabolic processes, for the regulation of ion transport, and for osmotic adjustment (Marschner, 1995). However, potassium concentrations in salt-stressed plants depend on whether the source of nitrogen fertilization is  $\text{NH}_4^+$  or  $\text{NO}_3^-$  as  $\text{K}^+$  uptake by cucumber seedlings salinized with NaCl was inhibited by the combination of both  $\text{NH}_4$  and  $\text{NO}_3$  but stimulated by  $\text{NO}_3^-$  alone; this response may be primarily associated with the well-documented competition between K and  $\text{NH}_4$  (Martinez and Cerda, 1989). Also, salt stress induced Na accumulation in New Zealand spinach and potassium content decreased with increasing salinity in New Zealand spinach (yousif *et al.*, 2010). High  $\text{K}^+/\text{Na}^+$  selectivity in plants under saline conditions has been suggested as an important selection criterion for salt tolerance (Ashraf, 2002; Wei *et al.*, 2003). While, Sheikhi and Ronaghi (2012) demonstrated that NaCl decreased potassium, iron and magnesium in spinach aerial parts but increased concentrations of N, phosphorus, sodium and chlorine.

Data presented in Table (4) established that application of humic acid and mycorrhiza revealed significant effect on the percentages of nitrogen, phosphorus, potassium, calcium and potassium/sodium ratio compared to control treatment in both seasons, except potassium in humic acid at 1.5 g/L, in the first season only. The obtained results indicated also that soil application treatments of humic acid and mycorrhiza, generally, decreased sodium contents. It is clear that addition of humic acid at 3.0 g/L + mycorrhiza gave the highest mean values of nitrogen, phosphorus, potassium, calcium and potassium/sodium ratio compared to the other treatments, in both seasons. However, the differences between (humic acid 1.5 g/l and mycorrhiza) and (humic acid at 3.0 g/L + mycorrhiza) in phosphorus percentage, were not significant, in the first season only. At humic acid of 3.0 g/L + mycorrhiza, the estimated percentage increase expressed as nitrogen, phosphorus, potassium and calcium, were (9.65 and 9.45%), (21.85 and 27.50 %), (7.42 and 7.36 %) and (16.46 and 13.61 %) in the first and second seasons, respectively and compare to the control treatment.



The current results could be attributed to the role of each ameliorative material. In this respect, humic acid enhanced uptake of N and  $\text{NO}_3^-$  and accelerated N metabolism by improving nitrate reductase activity, which resulted in production of protein in lettuce leaves (Haghighi *et al.*, 2012). Humic acid under different salt stress levels, slightly increased the content of N, P, K, while, Na content was decreased in pepper plants (El-Sarkassy *et al.*, 2017). Applications of humic acid to pepper plants grown under salt stress and normal conditions caused significant increases in N, P and K contents. (Akladios and Mohamed 2018). Aydin *et al.* (2012) showed that humic acid added to saline soil significantly increased plant nitrate, nitrogen and phosphorus content in bean (*Phaseolus vulgaris* L.).

Concerning mycorrhiza, Cantrell and Linderman (2001) found that inoculation of lettuce, grown under salt stress, with VA mycorrhizal fungi increased Ca, P, Zn, B, Cu and Mg than non-VAM plants at all salt levels. Similarly, Vicente-Sánchez *et al.* (2013) indicated that mycorrhization of plants increased the ability to acquire N, Ca, and K from both non-saline and saline media. Mycorrhiza

enhanced the contents of macronutrients such as N, P, K, Ca, and Mg of *Antirrhinum majus* under drought (Bati *et al.*, 2015). Mycorrhiza improve the uptake of almost all essential nutrients and contrarily decrease the uptake of Na and Cl, leading to growth stimulation (Evelin *et al.*, 2012). The interaction of salinity stress and AMF significantly affects the concentrations of P and N and the N:P ratio in plant shoots (Wang *et al.*, 2018). Concentrations of total P,  $\text{Ca}^{2+}$ , N,  $\text{Mg}^{2+}$ , and  $\text{K}^+$  were higher in the AMF-treated *Cucumis sativus* plants compared with those in the uninoculated plants under salt stress conditions (Hashem *et al.*, 2018).

The combined treatment between zero salinity and humic acid at 3.0 g/L + mycorrhiza, generally, achieved the highest average values of nitrogen, phosphorus, potassium and calcium and minimize the hazard effect of sodium in both seasons compared to the other treatments. Humic acid and mycorrhiza either alone or in combination under different salt stress levels, slightly increased the content of proline, N, P, K and photosynthetic pigments. while, Na content was decreased in pepper plants (El-Sarkassy *et al.*, 2017).

**Table (4):** Mean values of lettuce chemical contents as affected by salinity levels and soil application of humic acid and mycorrhiza and their interaction during winter seasons of 2019 and 2020.

Treatments	N (%)		P (%)		K (%)		Ca (%)		Na (%)		K/Na (%)		
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020	
<b>Salinity levels (mS/cm)</b>													
<b>Tap water</b>	3.63A*	3.57A	0.55A	0.58A	4.34A	4.35A	2.37A	2.35A	0.11D	0.15D	39.13A	41.20A	
<b>2.0</b>	3.38B	3.38B	0.51B	0.52B	3.90B	3.97B	2.18B	2.19B	1.46C	1.40C	2.94B	3.16B	
<b>4.0</b>	3.20C	3.18C	0.41C	0.42C	3.35C	3.40C	2.11C	2.04C	3.64B	3.50B	0.94C	0.98C	
<b>6.0</b>	2.74D	2.88D	0.33D	0.37D	3.21D	3.34D	1.80D	1.78D	4.58A	4.75A	0.72D	0.72C	
<b>Ameliorative treatments</b>													
<b>Control</b>	3.07D	3.07D	0.40D	0.40D	3.54D	3.58D	1.89C	1.91D	3.29A	3.34A	8.02C	10.36B	
<b>Humic 1 (H<sub>1</sub>)**</b>	3.23BC	3.20C	0.44C	0.46C	3.69CD	3.79C	2.14B	2.08C	2.25B	2.35B	14.21A	12.60AB	
<b>Humic 2 (H<sub>2</sub>)</b>	3.19C	3.27B	0.45B	0.49B	3.67C	3.81B	2.12B	2.12B	2.42B	2.42B	9.82C	9.02AB	
<b>Mycorrhiza (M)</b>	3.28B	3.29B	0.46B	0.49B	3.74BC	3.77BC	2.15B	2.12B	2.29B	2.23C	9.80BC	11.30AB	
<b>H<sub>1</sub> + M</b>	3.29B	3.31B	0.48A	0.50AB	3.77AB	3.80B	2.17AB	2.14B	2.23B	2.20C	10.76BC	11.98AB	
<b>H<sub>2</sub> + M</b>	3.37A	3.36A	0.48A	0.51A	3.80A	3.84A	2.21A	2.17A	2.20B	2.15C	12.97AB	13.82A	
<b>Water salinity levels × ameliorative treatments interaction</b>													
<b>Salinity levels</b>	<b>Ameliorative treatments</b>												
<b>Tap water</b>	<b>Control</b>	3.50d	3.34ghi	0.52de	0.53cd	4.24b	4.24d	2.28bcdef	2.26d	0.12g	0.11j	29.42c	38.78bc
	<b>Humic 1</b>	3.52cd	3.43ef	0.53cde	0.55bc	4.32ab	4.34c	2.51a	2.33bc	0.09g	0.10j	51.60a	45.26ab
	<b>Humic 2</b>	3.65abc	3.60bc	0.54cd	0.56b	4.34ab	4.35bc	2.34bcd	2.37ab	0.13g	0.37i	34.65bc	30.94c
	<b>Mycorrhiza</b>	3.67ab	3.64abc	0.56bc	0.59a	4.39a	4.38abc	2.35bc	2.38ab	0.13g	0.11j	34.29bc	40.08b
	<b>H<sub>1</sub> + M</b>	3.68ab	3.69ab	0.59a	0.61a	4.39a	4.39ab	2.38b	2.38ab	0.12g	0.10j	38.07b	42.60ab
<b>2.0 (mS/cm)</b>	<b>H<sub>2</sub> + M</b>	3.74a	3.70a	0.58ab	0.61a	4.39a	4.42a	2.37b	2.39a	0.10g	0.09j	46.74a	49.52a
	<b>Control</b>	3.18ef	3.11k	0.40g	0.41f	3.69e	3.66g	1.89j	2.01fg	2.66e	2.66g	1.40d	1.39d
	<b>Humic 1</b>	3.28e	3.33hij	0.51e	0.53cd	3.89d	4.06e	2.18gh	2.14f	1.10f	1.18h	3.53d	3.44d
	<b>Humic 2</b>	3.28e	3.37fgh	0.53cde	0.54bcd	3.91d	4.05e	2.22efgh	2.20e	1.29f	1.18h	3.06d	3.44d
	<b>Mycorrhiza</b>	3.48d	3.44ef	0.54cde	0.54bcd	3.91d	3.94f	2.24defg	2.24de	1.23f	1.19h	3.18d	3.33d
<b>4.0 (mS/cm)</b>	<b>H<sub>1</sub> + M</b>	3.47d	3.47de	0.54cde	0.55bcd	3.95cd	3.99f	2.25defg	2.25de	1.25f	1.15h	3.17d	3.48d
	<b>H<sub>2</sub> + M</b>	3.59cd	3.56cd	0.54cde	0.56bc	4.05c	4.10e	2.29bcde	2.27cd	1.23f	1.06h	3.30d	3.87d
	<b>Control</b>	3.08f	3.03kl	0.36hij	0.36ij	3.25hi	3.30l	1.79j	1.74k	4.62b	4.22c	0.71d	0.78d
	<b>Humic 1</b>	3.19ef	3.12k	0.38gh	0.39fgh	3.34fgh	3.39ijk	2.07i	2.09g	3.33d	3.46de	1.01d	0.98d
	<b>Humic 2</b>	3.16ef	3.12k	0.43f	0.44e	3.32fghi	3.43hi	2.13hi	2.09g	3.64cd	3.55d	0.92d	0.97d
<b>6.0 (mS/cm)</b>	<b>Mycorrhiza</b>	3.26e	3.24j	0.43f	0.44e	3.39f	3.40ij	2.18gh	2.08g	3.56d	3.32ef	0.95d	1.03d
	<b>H<sub>1</sub> + M</b>	3.23e	3.26ij	0.44f	0.45e	3.37fg	3.42hi	2.22efgh	2.10fg	3.33d	3.27ef	1.01d	1.04d
	<b>H<sub>2</sub> + M</b>	3.28e	3.29hij	0.44f	0.46e	3.41f	3.46h	2.26cdefg	2.13fg	3.33d	3.18f	1.02d	1.09d
	<b>Control</b>	2.53cd	2.80o	0.31kl	0.32k	2.96k	3.13m	1.61k	1.62l	5.74a	6.38a	0.54d	0.49d
	<b>Humic 1</b>	2.93g	2.91mn	0.35ij	0.36ij	3.22i	3.36jk	1.82j	1.77jk	4.50b	4.68b	0.72d	0.72d
<b>6.0 (mS/cm)</b>	<b>Humic 2</b>	2.66ij	3.00lm	0.29l	0.40fj	3.11j	3.42hi	1.81jj	1.82j	4.62b	4.57b	0.67d	0.75d
	<b>Mycorrhiza</b>	2.71i	2.83no	0.33jk	0.37hij	3.27ghi	3.34c	1.83j	1.79jk	4.25b	4.30c	0.77d	0.78d
	<b>H<sub>1</sub> + M</b>	2.76hi	2.82no	0.34j	0.38ghi	3.36fgh	3.39ijk	1.82j	1.82j	4.22b	4.28c	0.80d	0.79d
	<b>H<sub>2</sub> + M</b>	2.87gh	2.89mn	0.37ghi	0.41f	3.34fgh	3.38ijk	1.90j	1.88i	4.14bc	4.26c	0.81d	0.79d

Means having the same alphabetical letter (s) in column, within a comparable group of means, do not significantly differ, using the revised L.S.D. test at  $p = 0.05$  level of probability.

\*\*H<sub>1</sub>=Humic acid at 1.5 g/L, H<sub>2</sub>= Humic acid at 3.0 g/L, M= Mycorrhiza.

**Mean performances of chlorophyll and protein contents of lettuce**

Regarding the main effect of salinity levels on the total chlorophyll and protein percentage, data offered in Table (5) indicated that both tested parameters decreased as salinity levels increased in both seasons. The reduction rate on total chlorophyll and protein percentage varied depending on the level of imposed salinity stress. The highest values of total chlorophyll and protein percentage were obtained from control treatment, while that of 6.0 mS/cm salinity gave the lowest ones, in both seasons. At salinity of 6.0 mS/cm, the estimated percentage reductions, expressed as total chlorophyll and protein percentage, were (24.73 and 22.10 %), and (24.32 and 19.38 %) for the first and second seasons, respectively and relative to the control treatment. The reduction in photosynthetic rates in plants under salt stress is mainly due to the reduction in water potential (Chutipaijit *et al.*, 2011). Khan *et al.* (2013) indicated that total leaf

chlorophyll contents significantly decreased with an increasing in NaCl levels of cucumber plants. Likewise, Brengi (2019) found that increasing salinity levels from 2 to 4 dsm<sup>-1</sup> reduced significantly chlorophyll contents in cucumber plants. Also, with increasing salinity levels, total chlorophyll in pepper leaves significantly decreased, this reduction may be related to enhanced activity of the chlorophyll-degrading enzyme, chlorophyllase. Moreover, increased salt content also interferes with protein synthesis and influences the structural component of chlorophyll (Jaleel *et al.*, 2008). Similarly, in spinach, Seven and Sağlam (2020) reported that chlorophyll and protein contents were reduced as affected by salinity. Furthermore, increased salt content also delayed protein synthesis and influences the structural component of chlorophyll (Jaleel *et al.*, 2008). Recently, Gabr *et al.* (2022) in spinach plants, found that increasing salinity reduced total chlorophyll and protein percentage.

**Table (5):** Mean values of lettuce chlorophyll and protein percentage as affected by salinity levels and soil application of humic acid and mycorrhiza and their interaction during winter seasons of 2019 and 2020.

Treatments	Chlorophyll (SPAD)		Protein (%)		
	2019	2020	2019	2020	
<b>Salinity levels (mS/cm)</b>					
Tap water	40.22A*	41.78A	22.66A	22.29A	
2.0	37.33B	36.22B	21.13B	21.12B	
4.0	34.22C	33.72C	20.01C	19.85C	
6.0	31.33D	31.44D	17.15D	17.97D	
<b>Ameliorative treatments</b>					
Control	32.92C	32.33D	19.21D	19.20D	
Humic 1 (H <sub>1</sub> )**	36.17B	35.75C	20.19BC	19.98C	
Humic 2 (H <sub>2</sub> )	36.25B	36.17BC	19.92C	20.44B	
Mycorrhiza (M)	36.00B	36.33BC	20.50B	20.53B	
H <sub>1</sub> + M	36.33AB	36.75AB	20.54B	20.69B	
H <sub>2</sub> + M	37.00A	37.42A	21.06A	20.99A	
<b>Water salinity levels × ameliorative treatments interaction</b>					
<b>Salinity levels</b>	<b>Ameliorative treatments</b>				
Tap water	Control	39.67ab	32.33d	21.88d	20.88ghi
	Humic 1 (H <sub>1</sub> )	40.00a	35.75c	22.00cd	21.46efg
	Humic 2 (H <sub>2</sub> )	40.00a	36.17Bc	22.81abc	22.50bc
	Mycorrhiza (M)	40.33a	36.33Bc	22.94ab	22.73abc
	H <sub>1</sub> + M	40.67a	36.75Ab	22.98ab	23.06ab
	H <sub>2</sub> + M	40.67a	37.42a	23.38a	23.10a
	2.0 (mS/cm)	Control	35.00ef	41.00a	19.90ef
Humic 1 (H <sub>1</sub> )		37.33cd	42.00a	20.50e	20.81hij
Humic 2 (H <sub>2</sub> )		37.33cd	41.33a	20.48e	21.04fgh
Mycorrhiza (M)		37.67c	42.00a	21.73d	21.48ef
H <sub>1</sub> + M		38.33bc	42.33a	21.71d	21.71de
H <sub>2</sub> + M		38.33bc	42.00a	22.44bcd	22.23cd
4.0 (mS/cm)		Control	29.00j	33.67de	19.27f
	Humic 1 (H <sub>1</sub> )	34.00fg	36.00c	19.92ef	19.48k
	Humic 2 (H <sub>2</sub> )	36.00de	36.33bc	19.77ef	19.48k
	Mycorrhiza (M)	35.00ef	36.67bc	20.38e	20.23j
	H <sub>1</sub> + M	35.33ef	37.00bc	20.19e	20.38ij
	H <sub>2</sub> + M	36.00de	37.67b	20.52e	20.56hij
	6.0 (mS/cm)	Control	28.00j	28.33g	15.79j
Humic 1 (H <sub>1</sub> )		33.33g	33.00def	18.33g	18.17mn
Humic 2 (H <sub>2</sub> )		31.67hi	34.33d	16.60ij	18.75lm
Mycorrhiza (M)		31.00i	34.33d	16.96i	17.69no
H <sub>1</sub> + M		31.00i	36.00c	17.27hi	17.63no
H <sub>2</sub> + M		33.00gh	36.33bc	17.92gh	18.06no

Means having the same alphabetical letter (s) in column, within a comparable group of means, do not significantly differ, using the revised L.S.D. test at  $p = 0.05$  level of probability.

\*\*H<sub>1</sub>=Humic acid at 1.5 g/L, H<sub>2</sub>= Humic acid at 3.0 g/L, M= Mycorrhiza.

Data presented in Table (5) documented that application of humic acid and mycorrhiza revealed significant effect on the total chlorophyll and protein percentage compared to control treatment in both seasons. It is obvious that addition of humic acid at 3.0 g/L + mycorrhiza gave the highest mean values of total chlorophyll and protein percentage compared to the other treatments, in both seasons. However, the differences between (humic acid at 1.5 g/l and mycorrhiza) and (humic acid at 3.0 g/L + mycorrhiza) in total chlorophyll in both seasons and protein percentage in the first season were not significant. At humic acid of 3.0 g/L + mycorrhiza, the estimated percentage increase expressed as total chlorophyll and protein percentage, were (15.72 and 12.41%) and (9.63 and 9.32 %) in the first and second seasons, respectively and compare to the control treatment. The present results could be attributed to the role of each protecting material. In this respect, Humic acid had a positive effect on photosynthetic pigments of faba bean leaves (Dawood *et al.*, 2019). Applications of humic acid caused significant increases in chlorophyll a, chlorophyll b, [carotenoids](#) and total [photosynthetic pigments](#) content of pepper plants as compared with unstressed leaves (Akladious and Mohamed 2018). Cantrell and Linderman (2001) reported that chlorophyll content was linearly and more negatively affected by increasing salt levels than treating with mycorrhiza. [Hameed \*et al.\* \(2014\)](#) and [Talaat and Shawky \(2014\)](#) have observed that AMF-mediated enhancement in cytokinin concentration resulting in a marked photosynthetic translocation under salinity stress. In addition, AMF-mediated growth promotion under salinity stress was shown to be due to alteration in the polyamine pool ([Kapoor \*et al.\*, 2013](#)). Increasing salinity causes a reduction in chlorophyll content (Sheng *et al.*, 2008) due to suppression of specific enzymes that are responsible for the synthesis of photosynthetic pigments (Murkute *et al.*, 2006). A reduction in the uptake of minerals (e.g. Mg) needed for chlorophyll biosynthesis also reduces the chlorophyll concentration in the leaf (El-Desouky and Atawia, 1998). A higher chlorophyll content in leaves of mycorrhizal plants under saline conditions has been observed by various authors (Giri and Mukerji, 2004; Sannazzaro *et al.*, 2006; Zuccarini, 2007; Colla *et al.*, 2008; Sheng *et al.*, 2008). This suggests that salt interferes less with chlorophyll synthesis in mycorrhizal than in non-mycorrhizal plants (Giri and Mukerji, 2004). In the presence of mycorrhiza, the antagonistic effect of Na<sup>+</sup> on Mg<sup>2+</sup> uptake is counterbalanced and suppressed (Giri *et al.*, 2003). Inoculated plants under salt stress reach levels of photosynthetic capacity (estimated by chlorophyll content) even superior to those of non-stressed plants, showing that in this respect,

mycorrhization is capable of fully counterbalancing stress (Zuccarini, 2007).

The combined treatment of control salinity treatment (tap water) and humic acid at 3.0 gm/L + mycorrhiza, generally, attained the highest average values of total chlorophyll and protein percentage in both seasons, compared to the other treatments. The obtained results matching well with El-Sarkassy *et al.* (2017) who showed that humic acid and mycorrhiza either individual or in combination under different salt stress levels, slightly increased the content of proline, N, P, K and photosynthetic pigments. while, Na content was decreased in pepper plants.

The present results, generally, indicated that the application of humic acid and mycorrhiza might be significant treatment for successful lettuce plants and in elevating the salt hazard effects of salinity stress. The combined treatment of control salinity treatment (tap water) and humic acid of 3.0 g/L + mycorrhiza achieved the maximum values of the most studied parameters and might be considered as the best treatment for the production of high yield and good quality of lettuce plants under the environmental conditions of El Behiera Governorate and other similar regions.

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## المخلص العربي

## إمكانية التغلب علي أضرار الملوحة على نباتات الخس باستخدام حمض الهيوميك و الميكوريزا

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أجريت تجربتين في الأصص خلال الموسمين الشتويين المتتاليين لعامي 2019 و 2020 ، في مزرعة خاصة بقرية أحمد رامي ، منطقة البستان ، محافظة البحيرة ، مصر . الغرض الرئيسي لهذا العمل هو دراسة تأثير حمض الهيوميك والتلقيح بالميكوريزا تحت مستويات ملوحة مختلفة على النمو والخصائص الكيميائية لرؤوس الخس الصنف أيسبيرج . الهدف هو التوسع في زراعة الخس في المناطق المروية بمياه عالية الملوحة. تضمنت هذه التجربة 24 معاملة تمثلت في التوليفات بين أربعة مستويات ملوحة (ماء الصنبور ، 2,0 ، 4,0 و 6,0 ملليموز / سم) ، وستة معاملات تتمثل في ، خمسة معاملات معالجة محسنة (الميكوريزا ، حمض الهيوميك بتركيز 1,5 جم / لتر ، حمض الهيوميك بتركيز 3,0 جم / لتر ، حمض الهيوميك بتركيز 1,5 جم / لتر + الميكوريزا ، حمض الهيوميك بتركيز 3,0 جم / لتر + الميكوريزا) إضافة الى معاملة الماء المقطر كمعاملة كنترول. كان تصميم التجربة عبارة عن نظام القطع المنشقة في تصميم القطاعات العشوائية الكاملة ، حيث وزعت مستويات الملوحة في قطع الرئيسية ، بينما معاملات إضافات التربة بحمض الهيوميك والميكوريزا وزعت عشوائيا على القطع الفرعية. أظهرت النتائج المتحصل عليها بشكل عام أن جميع قيم الصفات المختبرة تتناقص مع زيادة مستويات الملوحة. يختلف معدل التخفيض على أي صفة تبعاً لمستوى إجهاد الملوحة المطبق. أظهر استخدام حامض الهيوميك و / أو الميكوريزا تأثيراً معنوياً في تحسين جميع الصفات المدروسة مقارنة بالمعاملة الكنترول في كلا الموسمين. حقق استخدام حمض الهيوميك بتركيز 3,0 جم / لتر + الميكوريزا أعلى متوسط قيم لعدد الأوراق ، و وزن الرأس ، والوزن الكلي الطازج ، و الوزن الجاف للرأس ، و طول الجذر ، و وزن الجذر الطازج ، و الوزن الجاف للجذر ، و النيتروجين ، و الفوسفور ، و البوتاسيوم ، و الكالسيوم ، و البروتين ، و الكلوروفيل الكلي ، و نسبة البوتاسيوم / الصوديوم ، و خفض محتوى الصوديوم مقارنة بالمعاملات الأخرى في كلا الموسمين. أعطت المعاملة المشتركة لحمض الهيوميك بتركيز 3,0 جم / لتر + الميكوريزا عند مستوى ملوحة ماء الصنبور أعلى متوسط قيم لمعظم الصفات المختبرة. اقترحت خاتمة هذا البحث إمكانية الاستفادة من الجمع بين حمض الهيوميك والميكوريزا لتعزيز نمو نباتات الخس وتقليل التأثير الضار للملوحة.

الكلمات المفتاحية: الخس ؛ الملوحة؛ الاجهاد الملحي ؛ حمض الهيوميك ؛ الميكوريزا ؛ النمو ؛ المحتوى الكيماوي