The impact of humic substances on oxidative stress and plant growth of spring barley exposed to NaCl

MARKETA JAROSOVA[1], BORIVOJ KLEJDUS[1,2], JOZEF KOVACIK[1,2], JOSEF HEDBAVNY[1]
[1]Department of Chemistry and Biochemistry
[2]CEITEC – Central European Institute of Technology
Mendel University in Brno
Zemedelska 1, 613 00 Brno
CZECH REPUBLIC

jarosoma@seznam.cz

Abstract: Humic acid is commercial product that contains many elements which improve the soil fertility and increase the biological availability of nutrients for plants. It consequently affects plant growth, yield and ameliorates the deleterious effects of salt stress. The impact of hydroponically applied humic acids on the growth and nutrient uptake of barley (*Hordeum vulgare*, Poaceae) plants grown at different salt concentrations was investigated. Sodium salts (chloride and sulphate) were added to distilled water to obtain 100 mM saline solutions. Humic acids were applied in different doses (0.01 and 0.1%) to the growing media with sodium salt or alone. Salinity negatively affected the plant growth and uptake of nutrient elements except Na and K. The interaction between applied salt and humic acids showed the increase of nutrient uptake. Humic acids represent the opportunity for the development of the agricultural productivity negatively affected by saline conditions.

Key-Words: salinity, humic substances, barley, plant growth parameter, nutrient content

Introduction

Salinity is the one of the major stressors limiting the agricultural production worldwide [1]. Salinity is caused by increased activity of soluble salts in the soil solution with electrical conductivity exceeding 4 $dS.m^{-1}$ [2]. Salinity is responsible for the plant cell dehydration, imbalance of water potential between cells and surrounding soil solution and turgor reduction. The intracellular osmotic potential can be reduced by the ability of plants to accumulate Na and Cl ions. High concentration of salt ions in cell leads to the inhibition of the mineral nutrient uptake [3], inactivation and degradation of cytoplasmatic enzymes and induction of low molecular highly hydrophilic organic substances. Ion concentration in cells can also affect a variety of enzymes involved in cell signaling, ion transport, energy metabolism (photosynthesis, ATP synthesis, respiration), carbohydrate and lipid metabolism, hormones (e. g. jasmonic acid, gibberelin, abscisic acid) and secondary metabolites synthesis [4, 5, 6, 7].

The opportunity for the improvement of agricultural production which can help to enhance the crop productivity and ensure the soil protection is the application of humic substances. This under-researched area of organic substances is a mixture of humin, humic acids, fulvic and hematomelanic acids. Humates are created by three different ways – from leonardite, peat or lake sapronel and lignosulphonate (so-called lignohumate) [8]. Lignohumates have a positive effect on photosynthesis, respiration intensity, root system formation, aboveground biomass development and seed germination [9, 10]. Further abilities of humic substances are the reduction of stress conditions induced by different abiotic stressors (e. g. unfavorable temperature, pH and salinity), the enhancement of nutrient uptake and the reduction of toxic metal intake from soil solution [9, 11, 12, 13].

The aim of the present study is to investigate the impact of humic acids to decrease the negative effects of sodium chloride on the growth and nutrient uptake of barley plants (*Hordeum vulgare* L., Poaceae) under salinity stress in controlled laboratory conditions.

Materials and Methods

Plant Material and Experimental Design

Barley seeds were germinated four days in water on Petri dishes with filter paper. Then, uniform plants were repotted to the small boxes (25 plants per box) with boiling beads and filter paper using different cultivation solutions under controlled laboratory conditions: 12 h day (8.00 am. to 8.00 pm.), photon flux density was 210 $\mu molm^{-2}\ s^{-1}$ at leaf level, 25/20°C day/night temperature and a relative humidity of ~60% [14].

For the experiments were used three different cultivars of spring barley – Bojos (B), Xanadu (X) and Radegast (R). Four-days old plants were exposed to five different treatments: (i) 100 mM NaCl, (ii) 0.01% humic acid (Sigma Aldrich, USA), (iii) 0.1% humic acid, (iv) 100 mM NaCl+0.01% humic acid and (v) 100 mM NaCl+0.1% humic acid. Simultaneously, three other experiments were performed: (i) with corrected pH to 4.0 or 7.0 under the same conditions as describes below, (ii) with using two different salts (100 mM NaCl and 100 mM Na_2SO_4) and pH (4.0 and 7.0) and (iii) cultivar Radegast exposed to 100 mM NaCl and humic acid (0.01 and 0.1%) originating from commercially available Lignohumate (Amagro, Czech Republic) and from Sigma Aldrich in technical quality. Results were compared to blank treatment where distilled water was used.

Plants were harvested after one week of exposure to these treatments. The fresh material was at first measured for root and shoots length, then dried on filter paper (in the case of roots) and considered. For the estimation of plant water content were samples dried at 75°C to constant weight (100-(dry mass x 100/fresh mass)). The quality of germination was monitored using germination energy ($n_{24}+n_{48}+n_{72}$), germination index ($(10(n_{24}+n_{48}+n_{72}))/(n_{24}+2n_{48}+3n_{72})$), mean germination time ($(n_{24}+2n_{48}+3n_{72})/\ n_{24}+n_{48}+n_{72})$) and germination rate ($(5n_{24}+3n_{48}+n_{72})/20$).

Quantification of Mineral Nutrients

Dry material was mineralized by mixture of concentrated ultra-pure HNO_3 and water in microwave extractor (Ethos Sel Microwave Extraction Labstation, Milestone Inc.) at 200 °C over 1 h. The clear mineralization outcome was quantitatively placed to plastic flasks and diluted to a final volume of 10 ml. Measurements were performed using an atomic absorption spectrometer AA30 (Varian Ltd., Mulgrave, Australia) and the air-acetylene flame. The mixture of HNO_3 and water used as a blank was also checked to ensure correctness of mineral quantifications [15, 16].

Statistical analyses

For the evaluation of significance of differences in parameters was used ANOVA followed by a Tukey's test (MINITAB Release 11, Minitab Inc., State College, Pennsylvania). Number of replications (*n*) in tables/figures denotes individual plants (root and shoot) measured for each parameter. Two independent repetitions were performed to ensure the reproducibility.

Results and Discussion

Humic acids are technically not a fertilizer, but they can be used as a suitable complement to organic and synthetic fertilizers. The application of humic substances along with fertilizer can reduce its required doses. Plant growth is dependent on the essential and non-essential nutrient uptake. Humic acids are able to chelate micronutrients and thus ensure their biological availability. Negative consequences of NaCl were described above. According to Khaled and Fawy [17], after the application of doses higher than 60 mM to reduction of calcium and potassium contents in corn occurs. The same reduction of mineral elements uptake was observed by Asik et al. [12] in wheat. Our analyzed data have shown that applied 100 mM NaCl increases the content of sodium ions (in pH 7.0) (Fig. 1). The accumulation of Na and Cl ions was also observed by Cimrin et al. [18]. The impact of lower pH in salt solution was observed. Acidity induced the enhancement of K^+ content. The same action was induced in neutral pH, when sodium ions were applied as sulphates. (Fig. 1).

Salinity decreased the maximal height of barley leaves (leaves and stems), which was by application of 100 mM NaCl reduced to 40%.

Dudeck et al. [19] found that plant aboveground biomass is more affected by NaCl effect than the root system, whose growth is not almost reduced. The impact of sodium chloride in neutral pH had not so visible effect on the leaf growth (Tab. 1). Salinity stress leads to the reduction of leaf water content (leaves with stems) which was also observed by Reina-Sánchez et al. [20]. Differences between NaCl- and Na2SO4-exposed plants were observed not only in the case of leaf height and total water content, but also in grain germination quality, which was monitored by germination energy, germination index, mean germination time and germination rate during three days. Acidic and neutral pH reduced the germination energy and other parameters, which were caltulated from the same input data, compared to control.

The most significant differences were observed in cultivar Radegast whose germination energy was higher than 90 % in control plants (Table 2).

Table 1 The impact of Na^+ salts on the aboveground biomass production and tissue water content of different cultivars of *Hordeum vulgare.*

		Control		100 mM NaCl		100 mM Na_2SO_4	
		pH 4.0	**pH 7.0**	**pH 4.0**	**pH 7.0**	**pH 4.0**	**pH 7.0**
BOJOS	**height (cm, *n=3*)**	13.2 ± 0.36^a	15.2 ± 0.60^a	6.23 ± 0.15^b	8.97 ± 0.40^c	13.4 ± 0.25^a	11.7 ± 0.26^b
	water content (%, *n=3*)	88.1 ± 3.49^a	84.9 ± 6.65^a	81.3 ± 6.85^{ab}	81.6 ± 4.65^a	75.9 ± 5.59^b	86.1 ± 5.22^a
XANADU	**height (cm, *n=3*)**	14.8 ± 0.74^a	16.2 ± 1.21^a	1.60 ± 0.53^c	9.87 ± 0.31^c	6.5 ± 1.01^b	13.3 ± 0.46^b
	water content (%, *n=3*)	77.9 ± 2.59^a	87.3 ± 6.17^a	63.7 ± 5.57^b	79.3 ± 3.01^a	69.3 ± 9.10^{ab}	87.2 ± 9.35^a
RADEGAST	**height (cm, *n=3*)**	14.2 ± 0.25^a	16.1 ± 0.35^a	3.37 ± 0.29^c	11.4 ± 0.75^c	10.7 ± 0.76^b	14.1 ± 0.72^b
	water content (%, *n=3*)	93.1 ± 2.60^a	96.7 ± 1.84^a	58.9 ± 3.95^b	79.9 ± 1.50^b	88.2 ± 2.70^a	82.3 ± 1.98^b

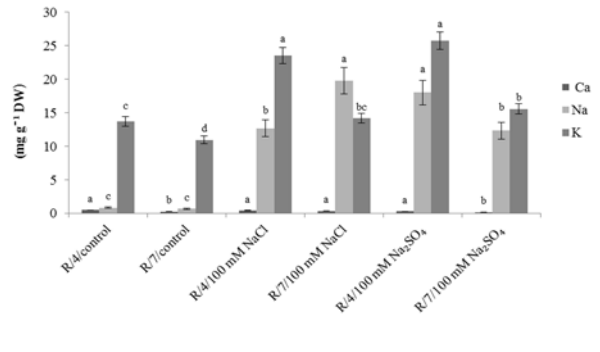
Data are means±SDs. Values within rows, followed by the same letter(s), are not significantly different according to Tukey's test ($P < 0.05$).

Table 2 Changes in germination quality of three barley cultivars exposed to different salt conditions.

	BOJOS						XANADU						RADEGAST					
	pH 4			pH 7			pH 4			pH 7			pH 4			pH 7		
	H_2O	NaCl	Na_2SO_4	H_2O	NaCl	Na_2SO_4	H_2O	NaCl	Na_2SO_4	H_2O	NaCl	Na_2SO_4	H_2O	NaCl	Na_2SO_4	H_2O	NaCl	Na_2SO_4
GE (%)	90.6	60.3	35.7	90.2	65.9	35.3	85.9	45.7	50.2	85.9	50.1	40.3	90.1	40.7	20.8	95.1	45.7	55.3
GI	5.81	4.14	4.38	6.20	4.33	5.38	6.29	4.09	4.54	6.07	3.84	3.80	6.42	4.21	5.10	5.58	3.91	3.67
MGT (d)	1.72	2.42	2.28	1.61	2.30	1.85	1.58	2.44	2.2	1.64	2.6	2.62	1.55	2.37	2.35	1.78	2.55	2.72
GR (%)	2.56	1.04	0.68	2.72	1.24	0.92	2.6	0.76	1.04	2.52	0.72	0.56	2.8	0.72	0.48	2.6	0.68	0.68

Sodium salts were applied as 100 mM NaCl and 100 mM Na_2SO_4 into the distilled water and subsequently pH was corrected to 4.0 or 7.0. The germination was observed 72 h (*n=3*). Legend: GE - germination energy, GI - germination index, MGT - mean germination time and GR - germination rate

Fig. 1 The accumulation of selected mineral nutrients in *Hordeum vulgare* leaves exposed to different Na^+ salts for one week (*n=3*). Data are means±SDs. Values for each parameter, followed by the same letter(s), are not significantly different according to Tukey's test ($P < 0.05$).



The most significant differences were observed in cultivar Radegast whose germination energy was higher than 90 % in control plants (Table 2). The application of salts reduced the observed germination parameters, regardless of the used pH. The cultivar Radegast was chosen as the most suitable object to prove the positive effect of humic acids in agricultural production negativelly affected by salinity to improve the germination, nutrient uptake ability and plant growth.

It was found that application of humic acids enhance the uptake of plant nutrients in 100 mM NaCl treatments. According Khan et al. [21] and Kurban et al. [22], small amounts of NaCl may cause a stimulative effect on the growth and nutrient uptake, but the increasing concentration have a toxic effects. In our study used concentration of NaCl caused the growth deceleration (Fig. 3). Many researchers studied the ability of NaCl to reduce the uptake of K^+ in plants due to the competetive process by Na^+ [23]. After the application of humic acids into the NaCl solution, the potassium content increased (Fig. 2). The content of Ca ions slightly decreased (Table 2) due to its antagonistic effect on the uptake of Na^+ [24]. In cultivars Bojos and Radegast are humic acids able to stimulate ion uptake. The ability of humic acids to enhance the nutrient uptake may be caused by fact that humic acids can interact with the phospholipid structures of the membranes and react as carriers of nutrients through them [17]. As well as Lee and Bartlett [25], with increasing concentration of humic acids the nutrient uptake does not increase (Fig. 2). We studied the impact of two types of humic acids. Humic acids in technical quality purchased from Sigma Aldrich induced the leaf growth in all treatments, but did not achieve the results set out in control plants. The same was found in the determination of water content (Table 3). The nutrient accumulation can be dependent on used humic substances. Although, the content of Na ions was not affected by the origin of humic acids, K^+ content increased after the addition of Lignohumate (Fig. 4).

Fig. 2 The accumulation of selected mineral nutrients in *Hordeum vulgare* leaves exposed to different treatments for one week (*n*=*3*). Three cultivars were studied – Bojos (Fig. 2A), Xanadu (Fig. 2B) and Radegast (Fig. 2C). Other details as in Fig. 1.

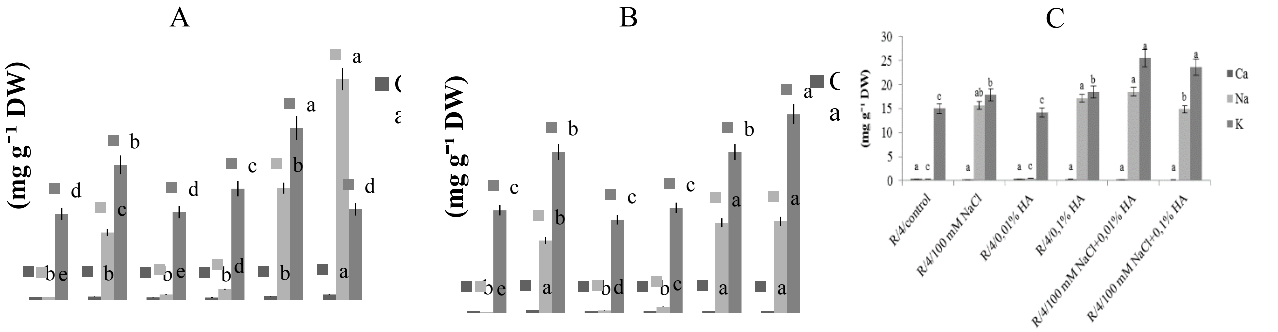
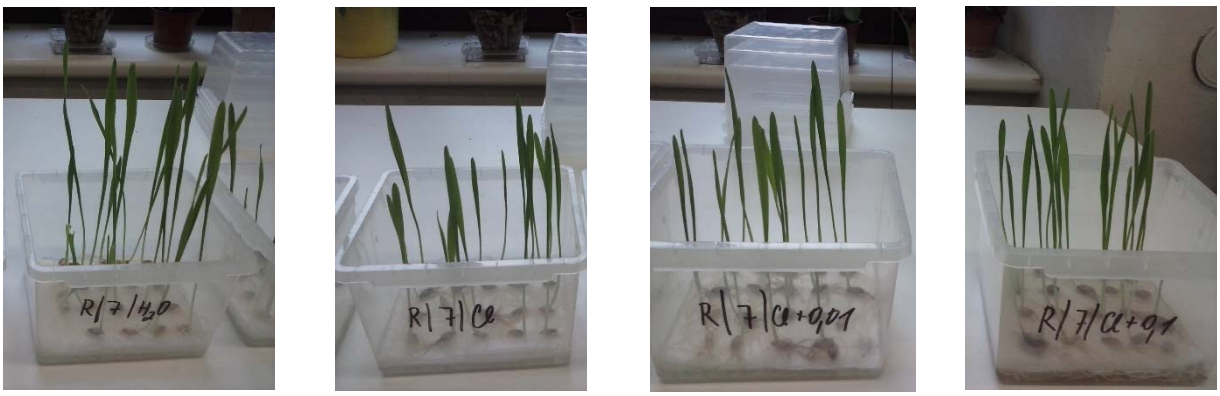


Table 3 The impact of using humic acid on the aboveground biomass production and the tissue water content in spring barley cv. Radegast.

		HA-L	HA-T
Control	**height (cm, *n=3*)**	16.4 ± 0.34^a	18.2 ± 1.23^a
	water content (%, *n=3*)	93.7 ± 2.27^a	94.1 ± 4.87^a
100 mM NaCl+0.01% HA	**height (cm, *n=3*)**	14.1 ± 0.19^b	15.8 ± 0.78^b
	water content (%, *n=3*)	89.2 ± 1.79^b	93.2 ± 3.59^a
100 mM NaCl+0.1% HA	**height (cm, *n=3*)**	14.3 ± 0.31^b	16.2 ± 1.04^b
	water content (%, *n=3*)	87.7 ± 3.01^b	90.5 ± 3.38^a

For the experiment were used two types of humic acids: (i) commercially available Lignohumate which contains >60 % humic acids from 10 % dry weight, (ii) commercially available humic acid in technical quality. Legend: HA-L – humic acid from Lignohumate, HA-T – humic acid in technical quality. Data are means±SDs. Values within rows, followed by the same letter(s), are not significantly different according to Tukey's test ($P < 0.05$).

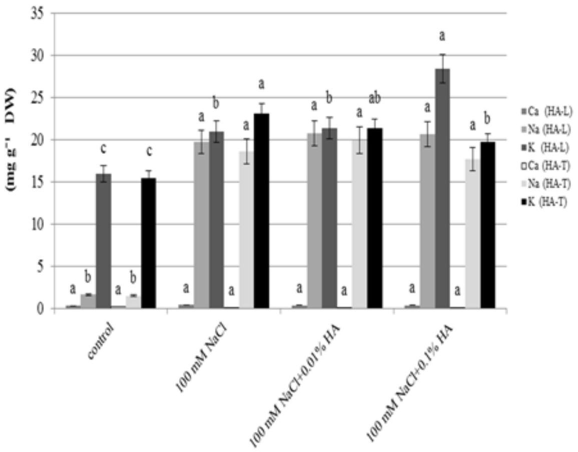
Fig. 3 The comparison of one-week old barley plants exposed to 100 mM NaCl alone (Fig. 3B) or in combination with 0.01% (Fig. 3C) and 0.1% humic acid (Fig. 3D) against the control (in distilled water) plants (Fig. 3A).



Conclusion

Humic substances can ameliorate negative soil properties; improve the plant growth and uptake of nutrients. It may be used in case of the negative effect of salt that would inhibit the plant growth and the uptake of nutrients. We found out that the applied doses and maybe also origin are important for taking benefits from humic acids under salt conditions. The application of humic acids offers a solution for the improvement of the agricultural production of salt sensitive plants and gives us the opportunity for further research.

Fig. 4 The accumulation of selected mineral nutrients in barley (cv. Radegast) leaves exposed to different treatments for one week (n=3). Two types of humic acids were used: (i) commercially available Lignohumate which contains >60 % humic acids from 10 % dry weight, (ii) commercially available humic acid in technical quality. Legend: HA-L – humic acid from Lignohumate, HA-T – humic acid in technical quality. Other details as in Fig. 1.



Acknowledgement

This work was realized in CEITEC - Central European Institute of Technology with research infrastructure supported by the project CZ.1.05/1.1.00/02.0068 financed from European Regional Development Fund. The work was also supported by OP Education for Competitiveness (European Social Fund and the state budget of the Czech Republic) CZ.1.07/2.3.00/30.0017 Postdocs in Biological Sciences at MENDELU and partially by Internal Grant Agency of Faculty of Agronomy, Mendel University (project IP 2/2014).

References:

[1] Munns R, Comparative physiology of salt and water stress. *Plant Cell Environ.,* Vol.25, 2002, pp. 239-250.

[2] Munns R, Genes and salt tolerance: Bringing them together. *Transley Rev. New Physiol.,* Vol.167, 2005, pp. 645-663.

[3] Marschner H, *Mineral Nutrition of Higher Plants*. 2nd. Edition. Academic Press. Inc. London, G.B., 2005, p. 446.

[4] Kosová K, Prášil IT, Vítámvás P, Protein contribution to plant salinity response and tolerance acquisition. *Int. J. Mol. Sci.,* Vol.14, 2013, pp.6757-6789.

[5] Jaarsma R, de Vries RSM, de Boer AH, Effect of salt stress on growth, Na^+ accumulation and proline metabolism in potato (*Solanum tuberosum*) cultivars. Plos One, Vol.8, 2013.

[6] Tsonev TD, Lazova GN, Stoinova ZG, Popova LP, A possible role for jasmonic acid in adapation of barley seedlings to salinity stress. *J. Plant Growth Reg.*, Vol. 17, 1998, pp. 153-159.

[7] Popova LP, Stoinova ZG, Maslenkova LT, Involvement of abscisic acid in photosynthetic process in Hordeum vulgare L. during salinity stress. *J. Plant Growth Reg.,* Vol.14, 1995, pp. 211-218.

[8] Pena-Mendez EM, Havel J, Patočka J, Humic substances-compounds of still unknown structure: applications in agriculture, industry, environment, and biomedicine. *J. Appl. Biomed*., Vol.3, 2005, pp. 13-24.

[9] Liu Ch, Cooper RJ, Humic acid application does not improve salt tolerance of hydroponically grown creeping bentgrass. J. *Amer. Soc. Hort. Sci.,* Vol.127, 2002, pp. 219-223.

[10] Garcia C, Hernandez T, Costa F, Ceccanti B, Dell´Amico C, Characterization of the organic fractions of an uncomposted and composted sewage sludge by isoelectric focusing and gel-filtration. *Biol. Fertil. Soils*, Vol.13, 1992, pp. 112-118.

[11] Masciandaro G, Ceccanti B, Ronchi V, Benedicto S, Howard L, Humic substances to reduce salt effect on plant germination and growth. *Comm. Soil Sci. Plant Anal.,* Vol.33, 2002, pp. 365-378.

[12] Asik BB, Turan MA, Celik H, Katkat AV, Effects of humic substances on plant growth and mineral nutrients uptake of wheat (*Triticum durum* cv. Salihli) under condition of salinity. *Asian J. Crop Sci.,* Vol.1, 2009, pp. 87-95.

[13] Ayuso M, Hernández T, Garcia C, Pascual JA, Stimulation of barley growth and nutrient absorption by humic substances originating from various organic materials. *Bioresource Technol.,* Vol.57, 1996, pp. 251-257.

[14] Kováčik J, Klejdus B, Hedbavny J, Štork F, Bačkor M, Comparison of cadmium and copper effect on phenolic metabolism, mineral nutrients and stress-related parameters

in *Matricaria chamomilla* plants. *Plant and Soil,* Vol.320, 2009, pp. 231–242.
[15] Kováčik J, Klejdus B, Štork F, Hedbavny J, Physiological responses of *Tillandsia albida* (Bromeliaceae) to long-term foliar metal application. *J. Haz. Mat.,* Vol.239–240, 2012, pp. 175–18.
[16] Kováčik J, Klejdus B, Babula P, Jarošová M, Variation of antioxidants and secondary metabolites in nitrogen-deficient barley plants. *J. Plant Physiol.,* Vol.171, 2014, pp. 260-2.
[17] Khaled H, Fawy HA, Effect of Different levels of humic acids on the nutrient content, plant growth, and soil properties under condition of salinity. *Soil and Water Res.*, Vol.6, 2011, pp. 21-29.
[18] Cimrin KM, Türkmen Ö, Turan M, Tuncer B, Phosphorus and humic acid application alleviate salinity stress of pepper seedling. *African J. Biotech.*, Vol.9, 2010, pp. 5845-5851.
[19] Dudeck AE, Peacock CH, Wildmon JC, Physiological and growth responses of St. Augustine cultivars to salinity. *HortScience*, Vol.28, 1993, pp. 46-48.
[20] Reina-Sánchez A, Romero-Aranda R, Cuartero J, Plant water uptake and water use efficiency of greenhouse tomato cultivars irrigated with saline water. *Agr. Water Manage*, Vol.78, 2005, pp. 54-56.
[21] Khan MA, Ungar IA, Showalter AM, Effects of salinity on growth, water relations and ion accumulation of subtropical perennial halophyte *Atripex griffhii* var. Stocksii. *Ann. Bot.,* Vol.85, 2000, pp. 225-232.
[22] Kurban H, Saneoka H, Nehira K, Adilla R, Premachandra GS, Fujita K, Effect of salinity on growth, photosynthesis and mineral composition in leguminous plant *Alhagi pseudoalhagi* (Bieb). *Soil Sci. Plant Nutr.,* Vol.45, 1999, pp. 851-862.
[23] Lopez MV, Satti SME, Calcium and potassium-enhanced growth and yield of tomato under sodium-chloride stress. *Plant Sci.*, Vol.114, 1996, pp. 19-27.
[24] Bernstein L, Effect of salinity and sodicity on plant growth. *Ann. Rev. Phytopathol.*, Vol.13, 1975, pp. 295-312.
[25] Lee YS, Bartlette RJ, Stimulation of plant growth by humic substances. *J. Am. Soil Sci. Soc.,* Vol.40, 1976, pp. 876-879.