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Effects of calcium nitrate and humic acid on pepper seedling growth under saline condition

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Abstract: *This study was carried out in order to determine effects of calcium nitrate and humic acid applications on pepper seedling growth under saline condition. A mixture of soil:sand (1:1) was used as a plant growing media including 90 mg kg^{-1} P_2O_5, 180 mg kg^{-1} K_2O and 250 mg kg^{-1} N. Four different doses of humic acid (0, 1000, 2000 and 4000 mg kg^{-1}) and calcium nitrate (0, 50, 100 and 150 mg kg^{-1}) were applied into the growing media. Before sowing Demre variety pepper seeds, 60 m mol NaCl was added into 300 cm^3 soil:sand mixture in each pot. Applications of humic acid and calcium nitrate significantly affected pepper seedling growth. 1000 and 2000 mg kg^{-1} humic acid and 50 mg kg^{-1} calcium nitrate applications increased fresh and dry leaf weight, fresh and dry root weight, stem diameter, root length and shoot length. The highest rates of humic acid (4000 mg kg^{-1}) and calcium nitrate (100 and 150 mg kg^{-1}) decreased these criteria of pepper seedling under the saline soil condition.*

Key words: *Pepper seedling, Salt stress, Humic acid, Calcium nitrate, Growth*
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Introduction

Soil salinity is one of the most important problems in dry and semi-dry climate areas of the world (Maas and Grattan, 1999). One-third of the world's arable land has been affected by soil salinity (Lazof and Berstein, 1999). Salts tend to accumulate in soil surface due to intensive evaporation conditions and insufficient leaching process (De Pascale and Barbieri, 1997). Accumulated salts deteriorate some soil physical and chemical properties. Excess exchangeable Na and high soil pH cause deformation of soil structure, decreases in hydraulic conductivity and infiltration rate of soils (Lauchli and Epstein, 1990). Crop productivity can be endangered by the negative changes in soil properties due to salinization.

Higher salt concentration in soil prevents plant growth (Mer *et al.*, 2000; Bhattacharjee, 2008; Ozdener and Kutbay, 2008) and growing plants can be died by the excess salt concentration (Donahue *et al.*, 1983). Salt stress in plants influence some basic plant metabolic process such as, photosynthesis, energy and lipid metabolism and protein synthesis (Parida and Das, 2005). It is known that photosynthetic reactions are influenced directly or indirectly due to the changes in structural organization and physicochemical properties of thylakoid membranes by salt stress (Karim *et al.*, 1999; Lichtenthaler *et al.*, 2005). Kim *et al.* (2009) found that increased endogenous gibberallins by jasmonic acid promoted plant growth such as dry weight of shoot and root in spinach plants under salt stress. They reported that NaCl inhibited reduction in dry weights was more decreased in root tissues than in shoot tissues.

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Humic substances such as humic acid, fulvic acid, are the major components (65-70%) of soil organic matter, increase plant growth enormously due to increasing cell membrane permeability, respiration, photosynthesis, oxygen and phosphorus uptake, and supplying root cell growth (Cacco and Dell Agnolla, 1984; Russo and Berlyn, 1990). It has been reported by many researchers that calcium has a positive effects on increasing plant tolerance to salts in saline soils (Ehret *et al.*, 1990). Calcium plays regulator role in metabolism (Cramer *et al.*, 1991). There was a competition between Na and Ca ions to enter into cell membrane. Therefore, it has been defended that higher calcium levels in soil protect cell membrane from negative effects of salinity (Busch, 1995). The objective of this study was to determine the effects of calcium and humic acid applications on pepper seedling growth under saline condition.

Materials and Methods

In this study, 1:1 ratio of soil:sand mixture was used as a plant growing media. Some properties of the growing media were determined using standard soil analyses methods (Kacar, 1994). The soil:sand mixture had a sandy loamy texture, non saline, slightly alkaline, low in organic matter and insufficient in phosphorus content (Table 1).

After filling the each pot with 300 cm^3 soil:sand mixture, 480 pots were autoclaved. Four doses of humic acid (HA_0:0, HA_1:1000, HA_2:2000, HA_3:4000 mg kg^{-1}) and $Ca(NO_3)_2$ (CaN_0:0, CaN_1:50, CaN_2:100, CaN_3:150 mg kg^{-1}) were applied into the pots with three replications. Each replication was formed from ten pots. As a basic fertilizer treatment 90 mg kg^{-1} P_2O_5, 180 mg kg^{-1} K_2O and 250 mg kg^{-1} N were also applied into each pot from Triple Super Phosphate

(TSP), K_2SO_4 and $(NH_4)_2SO_4$, respectively. Before pepper seedling, 60 mmol NaCl was added into 300 cm^3 soil:sand mixture in each pot. Pepper (*Capsicum annum cv* Derme) was used as a plant material. Three pepper seeds were sown to each pot and then the seedling were thinned to one. Experiment was carried out in a plant growth room of Horticultural Department in Yüzüncü Yil University under controlled conditions. The pots were placed in a growth chamber at 22±1°C with 8000 lux light intensity and the seedlings were irrigated with distilled water. There was not any leaching or drainage under the pots. The experiment was ended 8 weeks after the sowing. Shoot and root fresh weights (SFW and RFW), shoot and root dry weight (SDW and RDW), stem neck diameter (SND), root length (RL), shoot length (SL), leaf number (LN) of the seedlings were determined at the end of the study.

Variance analyses of the experimental data were accomplished in a completely randomized block design with two treatments ($Ca(NO_3)_2$ and humic acid) using SAS (1988) statistic program and significantly different means numbered according to Fisher's Least Significant Difference (LSD) test.

Results and Discussion

Variance analyses results for the growth criteria of pepper seedling are given in Table 2. Shoot fresh weight (SFW), root fresh weight (RFW), shoot dry weight (SDW), root dry weight (RDW), root length (RL), shoot length (SL) and leaf number (LN) were significantly influenced by the humic acid (HA) and $Ca(NO_3)_2$ (CaN) treatments. All these parameters, except shoot length (SL), were also affected by the HAxCaN interaction significantly. Stem neck diameter (SND) was only the parameter which was not affected by the treatments significantly.

Effects of humic acid and calcium treatments on the growth criteria and comparison of the means according to LSD test are given in Table 3. Humic acid application significantly increased shoot fresh weight and this increase diminished with increasing humic acid doses. While the highest mean SFW (3.80 g) was determined in HA_1, the lowest mean SFW (1.72 g) was in HA_0 treatment. The highest mean SFW (3.72 g) determined in CaN_1 was in the same range with the mean SFW (3.68 g) in CaN_0

Table - 1: Some properties of the growing media

Total salinity (%)	0.02	P (mg kg^{-1})	3.78
pH (1:2.5)	8.12	K (cmol kg^{-1})	0.43
Organic matter (%)	0.75	Ca (cmol kg^{-1})	28.0

treatment according to LSD test (Table 3). The lowest mean SFW was determined as 1.21 g in CaN_3 treatment. The highest and the lowest SFW values (5.31 and 0.82 g) were determined in HA_1xCaN_0 and HA_0xCaN_3 interactions, respectively (Table 3).

Increasing root fresh weight by the HA application was diminished by the higher application doses of HA. The highest and the lowest mean RFW values were determined in HA_1 (1.34 g) and HA_0 (0.59 g), respectively (Table 3). Mean RFW (0.73 g) in HA_3 was in the same group with mean RFW (0.59 g) in HA_0 statistically. Increasing CaN doses decreased the mean RFW. Mean RFW values ranged between 1.29 g in CaN_1 and 0.37 g in CaN_3. The highest and the lowest RFW values (1.90 and 0.22 g) were determined in HA_1xCaN_1 and HA_0xCaN_3 interactions, respectively.

Humic acid treatments significantly increased shoot dry weight. The highest and the lowest mean SDW values were 0.47 g in HA_2 and 0.20 g in HA_0, respectively (Table 3). Even though HA_3 treatment increased the SDW when compared with HA_0, they were in the same group statistically according to LSD test. While the highest mean SDW (0.44 g) was determined in CaN_1, the lowest mean SDW (0.14 g) was in the CaN_3 treatment. On the other hand, while HA_2xCaN_0 interaction gave the highest SDW (0.62 g), HA_3xCaN_3 interaction gave the lowest SDW (0.09 g). Increasing HA doses increased root dry weight significantly. Mean RDW values varied between the highest (0.14 g) in HA_2 and the lowest (0.06 g) in HA_0 (Table 3). The highest mean RDW (0.14 g) was determined in CaN_1 and the lowest mean RDW (0.05 g) was in the CaN_3 treatment. While HA_1xCaN_1 interaction had the highest RDW (0.22 g), HA_0xCaN_3 and HA_3xCaN_3 interactions showed the lowest RDW (0.02 g) values.

Mean stem neck diameter values in HA_1 (2.96 mm) and HA_2 (3.07 mm) were higher than mean SND values in HA_0 (2.46 mm) and HA_3 (2.53 mm) (Table 3). While the highest mean SND

Table - 2: Variance analyses for some growth criteria

Variation source	df	Shoot fresh weight		Root fresh weight		Shoot dry weight		Root dry weight	
		Mean square	F-value	Mean square	F-value	Mean square	F-value	Mean square	F-value
Humic Acid (HA)	3	12.06	64.1**	1.37	36.1**	0.202	81.6**	0.020	36.6**
$Ca(NO_3)_2$ (CaN)	3	16.91	89.8**	1.92	50.4**	0.219	88.5**	0.017	32.1**
HAxCaN	9	0.81	4.3**	0.09	2.3*	0.013	5.3**	0.002	3.3**
		Stem neck diameter		**Root length**		**Shoot length**		**Leaf number**	
Humic Acid (HA)	3	226159	1.0 ^{ns}	65.01	72.9**	30.18	60.1**	6.83	13.7**
$Ca(NO_3)_2$ (CaN)	3	223315	1.0 ^{ns}	68.27	76.6**	39.90	79.4**	23.36	46.7**
HAxCaN	9	225525	1.0 ^{ns}	3.13	3.5**	0.98	1.9 ^{ns}	1.17	2.3*

** significant at 0.01 and * at 0.05; ^{ns} not significant

Table - 3: The effects of humic acid and calcium nitrate treatments on growth criteria

	$Ca(NO_3)_2$	Humic acid (mg kg^{-1})				
	(mg kg^{-1})	0	1000	2000	4000	Mean*
	0	2.39	5.31	4.02	2.98	3.68 A
Shoot	50	2.03	5.08	4.70	3.08	3.72 A
Fresh	100	1.66	3.29	3.64	1.98	2.64 B
Weight (g)	150	0.82	1.50	1.66	0.84	1.21 C
	Mean*	1.72c	3.79a	3.50a	2.22b	
	0	0.80	1.68	1.14	0.92	1.14 AB
Root	50	0.75	1.90	1.47	1.02	1.29 A
Fresh	100	0.61	1.24	1.24	0.69	0.95 B
Weight (g)	150	0.22	0.53	0.47	0.27	0.37 C
	Mean*	0.59c	1.34a	1.08b	0.73c	
	0	0.26	0.44	0.62	0.23	0.41 A
Shoot	50	0.25	0.60	0.58	0.34	0.44 A
Dry	100	0.20	0.43	0.48	0.23	0.34 B
Weight (g)	150	0.10	0.19	0.18	0.09	0.14 C
	Mean*	0.20b	0.42a	0.47a	0.24b	
	0	0.07	0.11	0.16	0.08	0.11 B
Root	50	0.08	0.22	0.16	0.10	0.14 A
Dry	100	0.06	0.13	0.13	0.07	0.10 B
Weight (g)	150	0.02	0.06	0.09	0.02	0.05 C
	Mean*	0.06b	0.13a	0.14a	0.07b	
	0	2.55	3.22	3.42	2.78	2.99
Stem	50	2.70	3.26	3.50	2.91	3.09
Neck	100	2.49	2.97	3.18	2.46	2.78
Diameter(mm)	150	2.10	2.38	2.18	1.97	2.16
	Mean	2.46	2.96	3.07	2.53	
	0	10.25	14.47	15.30	10.60	12.65 A
Root	50	9.13	10.97	16.17	11.43	11.92 A
Lenght (cm)	100	8.50	10.02	13.23	7.75	9.87 B
	150	5.53	7.89	9.57	5.57	7.14 C
	Mean*	8.35c	10.84b	13.57a	8.84c	
	0	7.88	11.20	11.65	8.45	9.79 A
Shoot	50	7.00	10.83	10.92	8.72	9.37 A
Lenght (cm)	100	6.13	9.00	9.73	6.57	7.86 B
	150	4.92	6.34	6.60	5.18	5.76 C
	Mean*	6.48b	9.34a	9.72a	7.23b	
	0	11.80	12.10	12.03	12.10	12.01 A
Leaf	50	11.19	12.57	12.53	11.97	12.07 A
Number	100	10.13	12.05	12.30	9.43	10.98 B
	150	8.14	9.92	9.83	8.30	9.05 C
	Mean*	10.32b	11.66a	11.67a	10.45b	

* Means followed by different capital letters, in columns, and followed by different small letters, in lines, differ statistically at 0.01

(3.09 mm) was obtained in CaN_1, the lowest mean SND (2.16 mm) was in CaN_3 treatment. The highest mean values for root length (13.57 cm), shoot length (9.72 cm) and leaf number (11.67) were determined in HA_2 treatment (Table 3). HA_0 treatment gave the lowest mean values for RL (8.35 cm), SL (6.48 cm) and LN (10.32). While the highest mean values for RL (12.65 cm) and SL (9.79) were obtained in CaN_0 treatment, CaN_1 treatment gave the highest mean LN (12.07). The lowest mean numbers for RL (7.14 cm), SL (5.76 cm) and LN (9.05) were determined in CaN_3 treatment. While the highest values for RL (16.17 cm), SL (11.65 cm) and LN (12.57) were determined in HA_2xCaN_1, HA_2xCaN_0 and HA_1xCaN_1 interactions respectively, HA_0xCaN_3 interaction had the lowest values for RL (5.53 cm), SL (4.92 cm) and LN (8.14).

According to the results, 1000 mg kg^{-1} (HA_1) and 2000 mg kg^{-1} (HA_2) doses of humic acid generally had positive effects on pepper seedling growth. HA_1 and HA_2 treatments generally increased all growth criteria investigated in this study, except stem neck diameter. Even though HA_3 treatment slightly increased some growth criteria, effects of HA_3 on growth criteria were generally in the same group with the control (HA_0) treatment statistically. Ameliorative effects of humic acid on plant growth were reported by the most researchers (Böhme and Thi Lua, 1997; Türkmen *et al.*, 2005; Sonmez and Bozkurt, 2005). Masciandaro *et al.* (2002) found that using a soil seeded with maize in plant growth test, presented the best result when the mixture of saline solution–humic substances was used; while the worst plant performance was obtained with NaCl solution alone. Kulikova *et al.* (2005) reported that humic substances might show anti-stress effects under abiotic stress conditions such as, unfavorable temperature, pH, salinity etc. Humic substances could improve plant growth under soil condition with enhancing the uptake of nutrients and reducing the uptake of some toxic elements. David *et al.* (1994) have reported that humic substances promoted growth and more mineral nutrient uptake of plant due to the better-developed root systems. In this study, higher doses of HA had less effects on growth criteria in pepper seedling. Türkmen *et al.* (2004) similarly reported that 1000 g kg^{-1} of HA application positively affected plant growth under saline soil conditions, but higher doses of HA inhibited plant growth. Asik *et al.* (2009) determined that under salt stress, the lowest doses of both soil and foliar application of humic substances increased the nutrient uptake of wheat.

$Ca(NO_3)_2$ applications generally decreased growth criteria in this study. The first doses (50 mg kg^{-1}) of CaN significantly increased mean SDW and RDW. Even though CaN_1 treatment slightly increased mean values of the other growth criteria, effects of CaN_1 on growth criteria were generally in the same group with the control (CaN_0) treatment statistically. Ameliorative effects of $Ca(NO_3)_2$ on plant growth were reported by the most researchers (Al-Harbi, 1995; Turkmen *et al.*, 2002; Turkmen *et al.*, 2004). 100 and 150 mg kg^{-1} $Ca(NO_3)_2$ doses generally decreased growth criteria of pepper seedling. In this study, ameliorative effects of the lowest doses of CaN on growth criteria and the decreasing growth criteria by the higher doses of CaN could be explained with negative effect of higher $Ca(NO_3)_2$ doses. It has been known that calcium is also a salt resource for soils. Turkmen *et al.* (2004) similarly found that 100, 200 and 400 mg kg^{-1} Ca levels did not affected growth of tomato seedling significantly. In similar studies, it was reported that there were negative effects of higher Ca doses on growth criteria (Fraschina and Chiesa, 1993).

As a result, it was determined that under saline condition, lower doses (1000 and 2000 mg kg^{-1}) of humic acid had positive effects on pepper seedling growth, while higher doses of humic acid (4000 mg kg^{-1}) and $Ca(NO_3)_2$ (100 and 150 mg kg^{-1}) had negative effects on the plant growth in pepper seedling. Therefore, lower the humic acid dose applications, greater the plant growth due to possible positive effects of HA on nutrient uptake of plant under saline condition.

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