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Effects of Humic Substances on Plant Growth and Mineral Nutrients Uptake of Wheat (*Triticum durum* cv. Salihli) Under Conditions of Salinity

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Abstract: The effects of foliar and soil application of humic substances on plant growth and some nutrient elements uptake of wheat (*Triticum durum* Salihli) grown on various salt concentrations were examined. Sodium chloride was added to soil to obtain 15 and 60 mM saline conditions. The solid humus was applied to the soil one month before planting and the liquid humic acid was sprayed twice on the leaves on day 20 and 35 after seedling emergence. The application doses of solid humus were 0, 1 and 2 g kg^{-1} and the liquid humic acids were 0, 0.1 and 0.2%. Salinity negatively affected the growth of wheat; also decreased the dry weight and the uptake of nutrient elements except for Na and Mn. Soil application of humus increased the N uptake of wheat and foliar application of humic acid increased the uptake of P, K, Mg, Na, Cu and Zn. Although the effect of interaction between salt and soil humus application was found statistically significant, the interaction effect between salt and foliar humic acid treatment was not found significant. Under salt stress, the first doses of both soil and foliar application of humic substances increased the uptake of nutrients.

Key words: Foliar application, humic acid, humus, salinity, soil application

INTRODUCTION

Arid and semi-arid lands constitute approximately one third of the world's land surface (Arcihold, 1995) and salinity is the most important problem in these regions for agricultural production. About 9.5 billion ha of the world's soil are saline, except for large areas of secondarily salinized soil in cultivated land (Li *et al*., 2005). Salinity of soil is also becoming a major agricultural problem throughout some cereal growing basins in Turkey.

Plant growth and yield are reduced in salt-affected soil because of the excess uptake of potentially toxic ions (Grattan and Grieve, 1999). Soil salinity is characterized by high amounts of Na^+, Mg^{2+}, Ca^{2+}, Cl^-, HCO_3^-, SO_4^{2-} and B ions which have negative effects on plant growth. Generally, NaCl forms salt stress in nature. The general effect of soil salinity on plants is called a physiological drought effect. The high salt content decreases the osmotic potential of soil water and consequently, this reduces the availability of soil water for plants. Briefly, the uptake of water by plant roots is limited by increased amounts of Na and Cl. Eventually, high salt concentrations in the soil reduce the absorption of nutrients of the plants. Thus, this affects the fertility of the soil negatively.

It has known that fertility of soil is also related to soil organic matter content. Humic matter is the major component of soil organic matter. As organic materials in the soil decay, macromolecules of a mixed aliphatic and aromatic nature are formed (Chen and Aviad, 1990). The humic substances in the soil have multiple effects (Sangeetha *et al*., 2006). It may have both direct and indirect effects on plant growth (Chen and Aviad, 1990). Indirect effects involve improvements of soil properties such as

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aggregation, aeration, permeability, water holding capacity, micronutrient transport and availability (Tan, 2003). Direct effects are those, which require uptake of humic substances into the plant tissue resulting in various biochemical effects (Chen and Aviad, 1990).

Chen and Aviad (1990) and Varanini and Pinton (1995) summarized the effects of humic substances on plant growth and mineral nutrition, pointing out the positive effects on seed germination, seedling growth, root initiation, root growth, shoot development and the uptake of macro and microelements. Consequently, the use of humic substances has often been proposed as a method to improve crop production (Adani *et al*., 1998).

The agricultural areas affected by salt need amendments such as determination of the most suitable salt tolerant plant species (Abrol *et al*., 1988) or application of the different substances in order to reduce the effects of salinity (Lynch and Lauchli, 1985).

Kulikova *et al*. (2005) and Xudan (1986) also pointed out that humic substances might show anti-stress effects under abiotic stress (unfavorable temperature, pH, salinity, etc.) conditions. Humic substances may enhance the uptake of nutrients and reduce the uptake of some toxic elements. Therefore, it could be said that the application of humic substances could improve plant growth under the conditions of salinity. However, there are very few researches about humic acid application and its effect on the conditions of salinity (Masciandaro *et al*., 2002).

In this study, the effects of salinity, foliar and soil applications of humic substances on growth, mineral nutrients uptake of wheat and the comparisons of the soil and foliar applications of humic acid treatments at NaCl levels were investigated.

MATERIALS AND METHODS

The soil used in this study was collected from 0-20 cm depth of the field located in the Agricultural Research and Application Center of Uludag University. The soil was classified as Vertisol (*Typic haploxerert*) according to Soil Taxonomy and in the unit of Eutric Vertisol according to FAO/Unesco classification systems (Aksoy *et al*., 2001).

Some physical and chemical properties of the soil were analyzed; the texture was determined with the hydrometer method (Tan, 2005). pH and EC were measured in a 1:2.5 water extract, lime was determined according to Richards (1954). Organic matter content was analyzed according to the modified Walkley-Black method (Nelson and Sommers, 1982). Total nitrogen was determined by Buchi K-437 / K-350 digestion/distillation unit according to the Kjeldahl method (Bremmer, 1965). Available P was determined by Shimadzu UV 1208 model spectrophotometer according to the Watanabe and Olsen (1965). Exchangeable cations (Na, K, Ca and Mg) were extracted with ammonium acetate at pH 7.0 (Jackson, 1958) and determined by Eppendorf Elex 6361 model Flame photometer. Available Fe, Cu, Zn, Mn were extracted with DTPA (0.005M DTPA+0.01M $CaCl_2$+0.1M TEA pH 7.3) (Lindsay and Norwell, 1978) and determined by Philips PU9200x model Atomic Absorption Spectrophotometer. Some chemical and physical properties of the soil used in the research are shown in Table 1. The soil used in the experiment had sandy clay and neutral pH. It was low in terms of lime, salt and organic matter contents. The soil was not adequate in terms of nitrogen, phosphorus and zinc.

Table 1: Some chemical and physical properties of the soil used in the research

Texture class Sandy clay (%)									Exchangeable cations (mg kg^{-1})				Available microelements (mg kg^{-1})			
Clay	Silt	Sand	pH	EC (mS cm^{-1})	Lime $CaCO_3$ (%)	Org. C (%)	Total N (%)	Available P (mg kg^{-1})	Na	K	Ca	Mg	Fe	Cu	Zn	Mn
39.63	15.22	45.15	7.24	0.83	0.22	0.75	0.08	7.96	39.1	175.5	3860	282.0	5.56	1.3	0.2	10.4

The experiment was conducted in a greenhouse in completely randomized factorial design with three soil application doses of humus 0 (control) 1 and 2 g kg^{-1} three foliar application doses of humic acid (0, 0.1 and 0.2%) and three NaCl doses 0 (control), 15 and 60 mM. Each application consists of three replications. Soil applied humus was obtained from solid Deltahumus (65% w/w, pH 4.87, EC: 5.80 mS cm^{-1}) and foliar applied humic acid was obtained from liquid Deltahumat (humic acid: 12% w/v, pH 12.86, EC: 32.8 mS cm^{-1}) derived from leonardite which are the commercial products of Delta Chemicals Co.

Air-dried soil samples were passed through 4 mm sieve. For solid humus applications Deltahumus was put into a large bowl due to the application doses and the total weight of the soil was adjusted to 5 kg. The mixture was homogenized and put into polyethylene covered plastic pots. For foliar humic acid applications 5 kg of soil was put into polyethylene covered plastic pots. NaCl was added to the pots bought of soil and foliar treatments due to the application doses. The pots were exposed to 30 days of the incubation period. As a basal fertilizer, nitrogen (100 mg kg^{-1} as NH_4NO_3), phosphorus (80 mg kg^{-1}), potassium (100 mg kg^{-1} as KH_2PO_4), zinc (0.5 mg kg^{-1} as $ZnSO_4$) were applied to the pots before planting. Six durum wheat (*Triticum durum*) cultivar Salihli were grown in pots, which have 20 cm diameter and 18 cm depth. All pots was irrigated deionized water during with experiment. Deltahumat was sprayed twice in 5 L of deionized water, 20 and 35 days after seedling emergence as foliar treatment.

After two months vegetation period, plants were harvested, dried at 65°C, dry weights were determined and plant samples were wet digested by using HNO_3+$HClO_4$ (4:1) mixture. Nitrogen was determined by the Kjeldahl method (Bremmer, 1965) (Buchi K-437, K-350), P was determined by the Vanadomolybdophosphoric method (Kacar and İnal, 2008) (Shimadzu UV 1208), K, Na, Ca were determined by flame emission (Horneck and Hanson, 1998) (Ependorf Elex 6361) and Mg, Fe, Mn, Zn, Cu nutrients were determined by atomic absorption spectrometry (Hanlon, 1998) (Philips PU 9200x, Pye Unicam Ltd. GB).

All obtained data were subjected to statistical analysis. This analysis was performed by using Tarist, a statistical software (Tarist, 1994) and mean values were grouped with LSD multiple range test ($p<0.01$ and $p<0.05$).

RESULTS

Effects of NaCl on Plant Growth and the Uptake of Plant Nutrients

According to the analysis results, application of 15 mM NaCl increased the dry weight, N, P, K, Ca, Mg, Fe, Cu and Mn of the plants, but the amounts decreased with the application of 60 mM NaCl (Table 2). Particularly the effect of NaCl application at a dose of 60 mM had a negatively significant effect ($p<0.01$) on dry weight and mineral elements uptake of wheat.

Effects of Soil Application of Humus on Plant Growth and the Uptake of Nutrients

Soil applications of humus had a significant effect on the uptake of N ($p<0.05$) in wheat. When compared with the control treatment, the dry weight and mineral nutrients uptake of wheat were found

Table 2: Effect of NaCl to dry weight and plant nutrients uptake

NaCl (mM)	tdw. (g pot^{-1})	Nutrients uptake (mg tdw^{-1}) N	P	K	Ca	Mg	Na	Fe	Cu	Mn	Zn
Control	16.10a	321.2a	74.69a	416.3a	42.49a	50.73a	6.54c	1.34a	0.12a	0.32a	0.37b
15	18.09a	370.5a	82.69a	447.5a	42.64a	61.15a	76.58a	1.48a	0.16a	0.44a	1.08a
60	3.31b	85.6b	21.88b	70.59b	9.64b	11.95b	42.73b	0.24b	0.04b	0.15b	0.60ab
LSD	3.26	62.5	19.0	76.5	12.5	12.8	30.7	0.40	0.04	0.18	0.57
p<0.01	**	**	**	**	**	**	**	**	**	**	**

tdw: Total dry weight (g). **Significant at $p<0.01$. Values with common letter(s) are not significantly different higher at both application doses of humus (Table 3). The highest dry weight and the uptake of nutrients were obtained from 1 g humus kg^{-1} treatment. Dry weight and the uptake of nutrients were negatively affected from the application of 2 g humus kg^{-1}.

Effects of Foliar Application of Humic Acid on Plant Growth and the Uptake of Nutrients

Foliar applications of humic acid had a significant effect on dry weight and the uptake of mineral elements in wheat (Table 4). When compared with the control treatment, the dry weight and the uptake of nutrients were found higher in humic acid applications. Foliar application of humic acid affected the uptake of P which was statistically significant in the uptake of ($p<0.01$) Na, K, Cu and Zn ($p<0.05$) levels. However, its amounts were not found statistically significant on other nutrients. The highest dry weight and the uptake of nutrients were obtained from 0.1% dose of humic acid. Nevertheless, dry weight and the uptake of nutrients were decreased in 0.2% dose of humic acid, but the amounts except for Fe, Cu and Mn were found higher than control.

Interaction Effects Between Humic Substances and NaCl Treatment

Effects of foliar and soil application of humic substances on growth, the uptake of mineral nutrients and their interactions between salt doses were represented in Table 5. Interaction effect between soil humus and NaCl treatment was found statistically significant in the Mn ($p<0.01$) Zn and Na uptake ($p<0.05$) of wheat. The interactions in dry weight and the interaction of the amount of N, P, K, Ca, Mg, Fe and Cu in wheat were found to be insignificant.

Although, the application of NaCl decreased the dry weight and the uptake of nutrients application of soil humus limited the decrease especially in 60 mM treatment of NaCl, it was examined that the effect of soil application of humus generally enhanced the uptake of plant nutrients especially in 60 mM NaCl treatment. This was seen when compared with the other applications (Table 5).

The interaction effect between the foliar application of humic acid and NaCl was not found statistically significant. Foliar application of humic acid treatment enhanced the dry weight and the uptake of mineral elements in NaCl treatments. However, the highest dry weight and the uptake of nutrients were obtained from 0.1% treatment of humic acid in NaCl treatments.

Table 3: Effect of soil application of humic substance to dry weight and plant nutrients uptake

Treatment ($g\ kg^{-1}$)	tdw. ($g\ pot^{-1}$)	Nutrients uptake ($mg\ tdw^{-1}$) N*	P	K	Ca	Mg	Na	Fe	Cu	Mn	Zn
Control	16.68	313.6	78.74	414.53	48.36	53.01	7.87	1.18	0.11	0.33	0.38
1	19.80	427.8	110.98	654.82	62.94	71.52	10.70	1.88	0.16	0.59	0.49
2	19.43	356.3	91.62	481.05	50.14	60.64	8.03	1.54	0.14	0.40	0.47
LSD	ns	87.8	ns	ns	ns	ns	ns	ns	ns	ns	ns

*$p<0.05$, tdw: Total dry weight (g), ns: Not significant

Table 4: Effect of foliar application of humic acid to dry weight and plant nutrients uptake

Treatment (%)	tdw. ($g\ pot^{-1}$)	Nutrients uptake ($mg\ tdw^{-1}$) N	P**	K*	Ca	Mg*	Na*	Fe	Cu*	Mn	Zn*
Control	15.52	328.91	70.64	418.0	36.62	48.44	5.21	1.50	0.13	0.42	0.25
0.1	19.34	407.71	89.17	580.0	50.06	72.63	8.14	1.88	0.20	0.55	0.34
0.2	18.12	374.15	79.14	475.9	40.17	52.04	6.37	1.28	0.12	0.37	0.29
LSD	ns	ns	13.40	115.4	ns	17.20	2.21	ns	0.06	ns	0.06

**$p<0.01$, *$p<0.05$, tdw: Total dry weight (g), ns: Not significant

Table 5: Effect of soil and foliar application of humic substances to plant nutrients uptake under NaCl salt conditions

	Soil application (g kg^{-1})			Foliar application (%)		
NaCl (mM)	0	1	2	0	0.1	0.2
Dry weight (g pot^{-1})						
0	16.68	19.80	19.43	15.52	19.34	18.12
15	18.40	19.79	19.14	17.77	18.50	16.12
60	1.78	5.18	6.11	4.85	7.24	5.49
	HxS_{LSD} = ns			HxS_{LSD} = ns		
	Nutrients uptake (mg tdw^{-1})			Nutrients uptake (mg tdw^{-1})		
NaCl (mM)	0	1	2	0	0.1	0.2
Nitrogen						
0	313.6	427.8	356.3	328.91	407.71	374.15
15	359.9	432.5	351.7	381.09	363.12	343.78
60	58.0	134.0	142.5	113.35	158.09	115.52
	HxS_{LSD} = ns			HxS_{LSD} = ns		
Phosphorus						
0	78.74	110.98	91.62	70.64	89.17	79.14
15	90.72	92.12	88.21	74.65	79.76	73.65
60	17.75	33.06	39.15	26.02	39.32	31.83
	HxS_{LSD} = ns			HxS_{LSD} = ns		
Potassium						
0	414.53	654.82	481.05	418.06	580.00	475.97
15	470.35	507.10	448.96	424.58	458.01	360.87
60	48.35	116.56	125.91	92.82	152.04	102.92
	HxS_{LSD} = ns			HxS_{LSD} = ns		
Sodium						
0	7.87b	10.70c	8.03b	5.21	8.14	6.37
15	86.00a	92.35a	71.54a	67.15	66.29	63.33
60	26.25bB	61.01bA	76.27aA	59.21	82.40	59.47
	$HxS_{LSD<0.01}$ = 28.840			HxS_{LSD} = ns		
Calcium						
0	48.36	62.94	50.14	36.62	50.06	40.17
15	49.81	50.96	42.25	35.46	39.35	31.65
60	7.40	14.32	15.38	11.88	16.16	11.24
	HxS_{LSD} = ns			HxS_{LSD} = ns		
Magnesium						
0	53.01	71.52	60.64	48.44	72.63	52.04
15	65.66	59.02	61.38	56.63	61.82	45.64
60	8.55	16.74	20.58	15.36	24.24	14.67
	HxS_{LSD} = ns			HxS_{LSD} = ns		
Iron						
0	1.18	1.88	1.54	1.50	1.88	1.28
15	1.34	1.65	1.25	1.61	1.93	1.03
60	0.12	0.41	0.61	0.37	0.81	0.35
	HxS_{LSD} = ns			HxS_{LSD} = ns		
Copper						
0	0.11	0.16	0.14	0.13	0.20	0.12
15	0.16	0.17	0.16	0.16	0.18	0.12
60	0.02	0.05	0.06	0.05	0.05	0.04
	HxS_{LSD} = ns			HxS_{LSD} = ns		
Zinc						
0	0.38b	0.49a	0.47	0.25	0.34	0.29
15	0.54a	0.55a	0.45	0.34	0.39	0.34
60	0.11cB	0.25bAB	0.36A	0.20	0.26	0.19
	$HxS_{LSD<0.05}$ = 0.141			HxS_{LSD} = ns		
Manganese						
0	0.33b	0.59	0.40b	0.42	0.55	0.37
15	1.35a	0.95	0.77ab	0.82	0.99	0.81
60	0.30bB	0.96A	1.33aA	0.91	1.15	0.91
	$HxS_{LSD<0.01}$ = 0.649			HxS_{LSD} = ns		

tdw: Total dry weight (g). Capital letter(s) for each row and small letter(s) for each column for to compare the results

DISCUSSION

Little amounts of salt may cause a simulative effect on the growth and uptake of nutrients, but it shows toxic effect when the concentration rises. Khan *et al*. (2000) reported that the total dry weight accumulation of plant was not inhibited at low salinities, but dry weight production was significantly inhibited at high NaCl amounts. Kurban *et al*. (1999) also pointed out that the optimum growth of plants increased at low salinity but decreased at high salinity. The negative effect of salt on dry weight and the uptake of mineral elements can be attributed to the lower osmotic potential of the soil solution due to the increased concentration of NaCl. Many laboratory and glasshouse studies have shown that salinity can reduce N accumulation in plants (Alam, 1994), P concentrations (Navarro *et al*., 2001) and the uptake of K in plants due to the competitive process by Na (Lopez and Satti, 1996). High Na in soil solution also has an antagonistic effect on the uptake of Ca and Mg (Bernstein, 1975). This is most likely caused by displacing Ca in membranes of root cells (Yermiyahu *et al*., 1997). Furthermore, the reduced the uptake of mineral has been observed in several species of plants grown in saline conditions (Francois and Maas, 1999). In saline soil, the solubility of micronutrient is particularly low and plants grown in these soils often show deficiencies of this element (Page *et al*., 1990).

Chen and Aviad (1990), Fagbenro and Agboda (1993) and David *et al*. (1994) have reported promoted growth and nutrient uptake of plant due to the addition of humic substances. The plants take more mineral elements due to the better-developed root systems. In addition, the stimulation of ion uptake in applications with humic materials led many investigators to propose that these materials affect membrane permeability (Zientara, 1983). It is related to the surface activity of humic substances resulting from the presence of both hydrophilic and hydrophobic sites (Chen and Schnitzer, 1978). Therefore, the humic substances may interact with the phospholipids structures of cell membranes and react as carriers of nutrients through them.

The features that were discussed were negatively affected with the application of 2 g humus kg^{-1} level. This result might be related to levels of application. On the other hand, the application of the very high dose of humic acid is less effective (Lee and Bartlett, 1976). According to several researches, results were changing due to the levels of treatment, growing media and origin of humic substances (Chen and Aviad, 1990; Arancon *et al*., 2006).

There are a few researches on using humic substances as foliar application. Cooper *et al*. (1998) applied creeping bent grass in sand culture at rates of 100, 200 and 300 mg L^{-1} and they found that the rate of application did not have any effect on plant growth. Fernandez *et al*. (1996) pointed out that under field conditions, foliar application of leonardite extracts stimulated shoot growth and promoted the accumulation of K, B, Mg, Ca and Fe in leaves. However, when leaf N and leaf K values were below the sufficiency range, the foliar application of humic substances was ineffective to promote the accumulation of these nutrients in leaves. In a field experiment Govindasmy and Chandrasekaran (1992) sprayed humic acid extracted from lignite to sugarcane and they found that the addition of humic acid improved sugar yield and nutrient concentration in leaf blades and sheaths. Delfine *et al*. (2005) investigated the effect of foliar application of N and humic acid on growth and yield of durum wheat. Moreover, they specified that the foliar application of humic acid caused a transitional production of plant dry mass with respect to unfertilized control. In contrast to Delfine *et al*. (2005) and Pavlikova *et al*. (1997) studied the effect of potassium humate. Humic acid was applied by spray during the growth season of cultivated crops at a dose of 20 mg mL^{-1}. The yield of cultivated crops was not affected significantly by the application of potassium humate because of the high amounts of humic substances.

According to many researchers humic substances may enhance the uptake of some nutrients, reduce the uptake of toxic elements and could improve plant response to salinity. However, there are not many researches about humic acid application and its effects on plant salinity tolerance. Liu (1998) found out that the application of humic acid during salinity stress did not increase the uptake of N, P, K and Ca. Also, in present study; foliar application in 0.1% humic acid treatment increased the dry weight, N, P, K, Ca, Mg, Na, Fe, Zn and Mn amounts in plants in 60 mM NaCl when compared with control and 0.2% humic acid treatments. Chavan and Karadge (1980) also reported the increase of Mn contents in all the part of plant that have been treated with salt.

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 nutrient elements. Overall, we found out that the application doses are important for taking benefit from humic substances under salt conditions. Economical levels of application should be determinated and should not exceed 1 g humus kg^{-1} in soil and 0.1% in foliar.

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