Australian Journal of Basic and Applied Sciences, 3(2): 1266-1273, 2009
ISSN 1991-8178

Effects of Soil and Foliar Applications of Humic Substances on Dry Weight and Mineral Nutrients Uptake of Wheat under Calcareous Soil Conditions

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Abstract: A greenhouse research was conducted to determine the effects of soil and foliar applications of humic substances on dry matter and some nutrient elements' uptake of wheat grown under calcareous soil conditions. Agricultural lime was used to obtain five $CaCO_3$ doses (0, 5, 10, 20 and 40 %). The solid humus was applied to the soil at 0, 1 and 2 g kg^{-1} doses one month before planting and the liquid humic acid sprayed on the leaves at 0, 0.1 and 0.2 % doses at 20^{th} and 35^{th} days after seedling emergence. Although the increasing lime levels negatively affected the growth and mineral elements uptake of wheat, application of the soil humus limited this decrease especially under 20 % and 40 % doses of lime. The highest dry weight and nutrients uptake were obtained from 1 g kg^{-1} humus treatment. Foliar application of the Humic acid had statistically significant effect on Mg, Fe and Mn uptake. humic acid raised the dry weight and N, P, K, Ca, Mg, Na, Fe, Cu, Zn and Mn uptake of plants at non limed pots and the amounts were found high at 0.1 % dose of humic acid. The second dose (0.2 %) was found much more effective on dry weight and nitrogen uptake at high lime conditions.

Key words: Calcareous soils, foliar application, humic acid, plant growth, soil application, wheat

INTRODUCTION

The availability of plant nutrients are strongly related to the properties of soils. Calcareous soils are one of the most important factors that limit the nutrients' availability. Calcareous soils which are defined as having the presence of significant quantities of free excess lime ($CaCO_3$ or $MgCO_3$) are common in arid and semi-arid climates affecting over 600 million ha soils of the world (Leytem and Mikkelsen, 2005). These types of soils are also spread in Mediterranean areas and 58.62 % of the soils in Turkey comprising lime more than 5 % (Eyupoglu, 1999). These soils are also important for production of field crops, especially 9.4 million ha wheat sowing areas in Turkey. Micronutrients deficiency is one of the most important abiotic stresses in plants grown on calcareous soils (Xudan, 1986; Kulikova, *et al.,* 2002). In addition, increased nitrogen (N) losses as ammonia and reduced solubility of phosphorus (P) occur in these types of soils. Thus, negatively affects the soil fertility.

It has known that fertility of soils is also related to soil organic matter content. Humic substances (humic and fulvic acids) are the major components of soil organic matter and the term of "humus" is widely accepted as synonymous for the soil organic matter (Chen and Aviad, 1990). The humic substances in the soil have multiple effects that can greatly benefit plant growth (Lobartini, *et al.,*1997; Tan, 1998; Nardi, *et al.,*2002;

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Cimrin and Yilmaz, 2005; Sangeetha *et al.,* 2006). It may have both direct and indirect effects on the plant growth (Chen and Aviad, 1990). Indirect effects involve improvements of the soil properties such as 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; Nardi, *et al.,*2002; Escobar, *et al.*, 1996).

The effects of humic substances on plant growth, under conditions of adequate mineral nutrition, consistently show positive effects on plant biomass (Chen and Aviad, 1990). These substances affect the solubility of many nutrient elements by building complex forms or chelating agents of humic matter with metallic cations (Lobartini, *et al.,* 1997). Recent studies on the subject summarize the effects of humic substances on plant growth and mineral nutrition, underlining, above all positive effects on seed germination, seedling growth, root initiation, root growth, shoot development and the uptake of some macro (e.g. K, Ca, P) and microelements (e. g. Fe, Zn, Mn) (Chen and Aviad, 1990; Nardi, *et al.,* 2002; Varanini and Pinton, 1995;Bohme and Thi Lua, 1997; Eyheraguibel, *et al.,* 2008). As a consequence, the use of humic substances has often been proposed as a method to improve crop production (Adani, *et al.,* 1998). The effects of humic acid derived from various organic wastes on seedling growth of tomato in some growth media were investigated by David *et al.,* (1994), Loffredo *et al.,* (1997), Pertuit *et al*., (2001), and Atiyeh *et al*., (2002). The positive effects of the humic substances were also observed on the studies such as dry matter yield increases on corn and oat seedling (Lee and Bartlett, 1976; Albuzio, *et al.,* 1994; Celik, *et al.,* 2008), yield increases on radish and green bean seedings (Singhvi, 1989; Russo and Berlyn, 1992), Although in many researches, the effects of humic substances on numerous plants such as tomato (Bohme and Thi Lua, 1997; Adani, *et al.,* 1998; Padem and Ocal, 1999), forage turnip (Albayrak and Carnas, 2005), spinach (Ayas and Gulser, 2005), bentgrass (Cooper, *et al.,* 1998), blackgram (Natesan, *et al.,* 2006) have been well documented, the growth response of plants has not been adequately studied under abiotic stress conditions. Under the calcareous soil conditions, application of the humic substances may increase the tolerance of the plants to stress, and promote growth by increasing nutrients uptake (Tan, 2003). The purpose of this work was to determine the effects of foliar and soil application of humic substances to growth and nutrient uptake of wheat grown on calcareous soil conditions.

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 (Soil, 1951). pH and electrical conductivity (EC) were measured in 1:2.5 water extract, lime was determined according to Richards (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 Olsen method (Olsen, *et al.,* 1954). Exchangeable cations sodium, potassium, calcium and magnesium (Na, K, Ca and Mg) were extracted with ammonium acetate at pH 7.0 (Pratt, 1965) and determined by Eppendorf Elex 6361 model Flame photometer. Available iron, copper, zinc and manganese (Fe, Cu, Zn, Mn) were extracted with DTPA (0.005M DTPA (Diethylene triamine pentaacetic acid) + 0.01M $CaCl_2$ + 0.1M TEA (Triethanolamine) pH 7.3) (Lindsay and Norvell, *et al.,* 1978) and determined by Philips PU9200x model Atomic Absorption Spectrophotometer. Some chemical and physical properties of the soil used in the research are given in Table 1.

The experiment was conducted in greenhouse, in completely randomized factorial design with three soil application doses of humus (0, 1 and 2 g kg^{-1}), three foliar application doses of humic acid (0, 0.1 and 0.2 %) and five $CaCO_3$ doses (0 (control), 5, 10, 20 and 40 %). Each application consists of three replications. Humic substances were derived from leonardite (a naturally occurring, highly compressed and decomposed, soft brown and coal-like organic material, usually found in conjunction with deposit of lignite). 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 (12 % w/v, pH: 12.86, EC: 32.8 mS cm^{-1}) which are the commercial products of Delta Chemicals Co.,

Air-dried soil samples were passed through 4 mm-sieve. Solid humus and $CaCO_3$ 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. The pots exposed to 30 days incubation period. As 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* Salihli spp.) plants were grown in pots, which have 20 cm diameter and 18 cm depth. Humic acid was sprayed twice in 5 litters of deionized water 20 and 35 days after seedling emergence as foliar treatment.

After a two month vegetation period, plants were harvested, dried at 70ºC, dry weights were determined and plant samples were wet digested by using HNO_3+$HClO_4$ mixture. Nitrogen was determined by the Kjeldahl method (Bremmer, 1965) (Buchi K-437, K-350), P was determined by the vanadomolybdophosphoric method (Lott, *et al.,* 1956) (Shimadzu UV 1208), K, Na, Ca were determined by flame emission (Horneck and Hanson, 1998) (Ependorf Elex 6361), Mg, Fe, Mn, Zn and 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 $p< 0.05$).

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

Properties	Quantities
Texture	Sandy clay
Sand, %	45.15
Silt, %	15.22
Clay, %	39.63
pH	7.24
EC, mS cm^{-1}	0.83
Lime, % $CaCO_3$	0.22
Organic matter, %	1.30
Total nitrogen (N), %	0.08
Available phosphorus (P), mg kg^{-1}	7.96
Exchangeable Cations, me $100g^{-1}$	
Sodium (Na)	0.17
Potassium (K)	0.45
Calcium (Ca)	19.26
Magnesium (Mg)	2.35
Available microelements, mg kg^{-1}	
Iron (Fe)	5.56
Copper (Cu)	1.30
Zinc (Zn)	0.20
Manganese (Mn)	10.44

RESULTS AND DISCUSSION

Effects of soil application of humus on plant growth and on the uptake of nutrients:

Effects of soil application of humus on growth, mineral nutrients uptake and their interactions between lime levels were given in Table 2 and 3. According to the analysis results, increasing lime levels had negative effect on dry weight and mineral elements uptake of the wheat especially at 20 and 40 % doses of lime (Table 2 and 3). The soil application of humus had statistically significant effect ($p<0.01$) on the dry weight and mineral elements uptake except Zn and Na. When compared with the control treatment, the dry weight and nutrient elements uptake of the plants were found high at humus applications (Table 2 and 3). The highest dry weight and nutrients uptake were obtained from 1 g kg^{-1} of humus treatment and the amounts tend to decrease on 2 g kg^{-1} of humus application.

Interaction effect between the soil humus and lime was found statistically significant on Fe ($p<0.01$) and on dry weight, N, Cu and Mn uptake ($p<0.05$), except for K, Ca, Mg, Na, and Zn. Although the application of lime decreased the dry weight and nutrients uptake, application of the soil humus limited the decrease especially under 20 % and 40 % doses of lime. 1 g kg^{-1} dose of the humic acid treatments gave affirmative results on dry weight and P uptake (Table 2) at these lime levels. Fe, Cu and Mn uptake of the wheat gave the same results as dry weight and P (Table 3). Although soil application of humus generally enhanced N, K, Na, Ca, Mg and Zn uptake when compared to control, this effect was not found statistically significant.

Effects of foliar application of humic acid on plant growth and on the uptake of nutrients:

Increasing lime levels had also negative effect on dry weight and mineral elements uptake of wheat grown on calcareous soil conditions (Table 4 and 5). Dry weight, N, K, Ca, Na, Mg, Cu, Mn and Zn uptake of the plants showed a little raise at 5 % of lime but decreased with increasing doses especially on 20 and 40 % doses of lime. Foliar application of the humic acid had statistically significant effect on Mg, Fe and Mn uptake ($p<0.01$) (Table 5). Although the dry weight and other nutrients were also affected, they were not found statistically significant. Interaction effect between foliar application of the humic acid and lime was found statistically significant on dry weight, P, Na, Mg and Fe ($p<0.05$), Cu and Zn uptake ($p<0.01$). When compared with the control treatment, foliar application of humic acid raised the dry weight and N, P, K, Ca, Mg, Na, Fe, Cu, Zn and Mn uptake of plants at non limed pots and the amounts were found high at the first dose of humic acid (0.1 %). The first dose of humic acid also found effective on low lime doses. The second dose of humic acid (0.2 %) was found much more effective on dry weight and nitrogen uptake at 20 and 40 % levels of lime.

Table 2: Effects of soil application of humus on dry weight of wheat under increasing lime levels (g pot^{-1}).

	Humus treatments, (H)			
Lime (L),%	H_0control	$H_1$1 g kg^{-1}	$H_2$2 g kg^{-1}	Means
0	16.68 B ab	19.80 A a	19.43 A a	18.64 a
5	18.49 A a	17.69 A b	19.19 A ab	18.46 ab
10	16.10 A b	17.23 A b	17.28 A b	16.87 b
20	12.69 B c	16.59 A b	15.20 A c	14.83 c
40	11.86 B c	16.44 A b	14.53 A c	14.28 c
Means	15.16 B	17.55 A	17.13 A	

$H_{LSD<0.01}$=1.222, $L_{LSD<0.01}$= 1.592, $HxL_{LSD<0.05}$= 2.036

The differences between values indicated by different letters are significant. Capital letters indicate rows and small letters indicate columns

Table 3. Effects of soil application of humus on macro and micro nutrients uptake of wheat under increasing lime levels

Lime (L), %	Humus treatments (H), g kg^{-1}							
	H_0	H_1	H_2	Means	H_0	H_1	H_2	Means
		Nitrogen, mg tdw^{-1}				Sodium. mg tdw^{-1}		
0	313.50 B b	427.50 A a	356.60 A a	365.87 a	7.8	11.23	8.4	9.14 a
5	384.40 A a	385.57 A ab	371.44 A a	380.57 a	9.24	7.13	7.68	8.02 ab
10	331.72 A b	369.67 A b	335.66 A a	345.67 a	6.99	6.94	6.91	6.95 ab
20	234.51 B c	349.75 A b	266.79 A b	283.68 b	5.44	6.10	6.10	5.88 c
40	199.19 C c	299.64 A c	249.17 B b	249.34 b	3.93	7.14	6.31	5.79 c
Means	292.67 B	366.49 A	315.93 B		6.68	7.71	7.08	
$H_{LSD<0.01}$=32.54, $L_{LSD<0.01}$= 38.32, $HxL_{LSD<0.05}$=48.89					H_{LSD}=ns, $L_{LSD<0.01}$= 2.71, HxL_{LSD}= ns			
		Phosphorus, mg tdw^{-1}				Iron. mg tdw^{-1}		
0	78.54 B a	110.95 A a	91.11 B a	93.53 a	1.17 B a	1.88 A ab	1.54 Ababc	1.53
5	80.69 A a	79.06 A b	77.17 A a	78.97 b	1.31 A a	1.40 A bc	1.80 A a	1.50
10	63.80 A b	63.24 A c	48.96 B b	58.67 c	1.09 A a	1.24 A c	1.63 A ab	1.32
20	37.11 AB c	47.04 A d	32.70 B c	38.95 d	0.86 B a	2.24 A a	1.00 B c	1.37
40	22.12 A d	34.51 A d	24.74 A c	27.12 e	1.04 A a	1.44 A bc	1.04 A bc	1.17
Means	56.45 B	66.96 A	54.94 B		1.09 B	1.64 A	1.40 AB	
$H_{LSD<0.01}$=8.35, $L_{LSD<0.01}$= 10.99, $HxL_{LSD<0.05}$= 14.05					$H_{LSD<0.01}$=0.33, L_{LSD}= ns, $HxL_{LSD<0.01}$= 0.61			
		Potassium, mg tdw^{-1}				Copper, mg tdw^{-1}		
0	414.78	655.08	480.93	516.9 a	0.11 B ab	0.16 A a	0.14 A a	0.137 a
5	499.6	472.49	509.58	493.9 ab	0.12 A a	0.11 A c	0.13 A ab	0.120 b
10	429.9	492.56	431.72	451.4 abc	0.09 A b	0.13 A bc	0.13 A ab	0.117 b
20	321.52	495.83	385.42	400.9 bc	0.08 B b	0.13 A abc	0.12 AB ab	0.110 b
40	270.06	433.38	377.59	360.3 c	0.08 B b	0.14 A ab	0.10 B b	0.107 b
Means	387.17 B	509.87 A	437.05 AB		0.097 B	0.132 A	0.124 B	
$H_{LSD<0.01}$=90.33, $L_{LSD<0.01}$= 96.61, HxL_{LSD}= ns					$H_{LSD<0.01}$=0.018, $L_{LSD<0.05}$= 0.019, $HxL_{LSD<0.05}$= 0.034			
		Calcium, mg tdw^{-1}				Zinc, mg tdw^{-1}		
0	48.35	63.4	49.83	53.86 a	0.38	0.49	0.46	0.443
5	50.52	52.47	50.45	51.15 ab	0.39	0.34	0.44	0.390
10	46.12	56.5	48.38	50.33 ab	0.29	0.36	0.30	0.317
20	35.47	59.34	42.57	45.79 bc	0.24	0.44	0.26	0.313
40	33.24	52.71	46.16	44.04 c	0.28	0.66	0.25	0.397
Means	42.74 B	56.88 A	47.48 B		0.320	0.460	0.340	
$H_{LSD<0.01}$=7.89, $L_{LSD<0.05}$= 6.02, HxL_{LSD}= ns					H_{LSD}=ns, L_{LSD}= ns, HxL_{LSD}= ns			
		Magnesium, mg tdw^{-1}				Manganese, mg tdw^{-1}		
0	53.51	71.38	60.75	61.88 a	0.33 B a	0.60 A a	0.40 B c	0.443
5	59.16	55.38	59.29	57.94 ab	0.36 B a	0.45 AB a	0.61 A ab	0.473
10	54.17	58.72	51.84	54.91 abc	0.36 A a	0.48 A a	0.43 A bc	0.423
20	40.9	60.44	51.15	50.83 bc	0.24 B a	0.49 A a	0.33 AB c	0.353
40	36.37	60.31	43.73	46.80 c	0.22 B a	0.48 A a	0.64 A a	0.447
Means	48.82 B	61.25 A	53.35 B		0.303 B	0.499 A	0.482 A	
$H_{LSD<0.01}$= 6.83, $L_{LSD<0.01}$= 9.98, HxL_{LSD}= ns					$H_{LSD<0.01}$=0.088, L_{LSD}= ns, $HxL_{LSD<0.05}$= 0.181			

The differences between values indicated by different letters are significant. Capital letters indicate rows and small letters indicate columns
tdw: total dry weight

Table 4: Effects of foliar application of humic acid on dry weight of wheat under increasing lime levels (g pot^{-1}).

Lime (L),%	Humic acid treatment (HA)			
	H_0control	$H_1$0.1 %	$H_2$0.2 %	Means
0	15.52 B ab	19.34 A a	18.12 AB a	17.66 a
5	17.19 A a	15.04 AB b	13.74 B b	15.32 ab
10	14.69 A ab	14.61 A b	14.37 A b	14.56 bc
20	13.53 AB bc	12.45 B b	16.55 A ab	14.18 bc
40	11.42 A c	11.92 A b	13.67 A b	12.34 c
Means	14.47	14.67	15.29	

HA_{LSD}= ns, $L_{LSD<0.01}$= 2.517, $HAxL_{LSD<0.05}$= 3.219

The differences between values indicated by different letters are significant. Capital letters indicate rows and small letters indicate columns

Table 5: Effects of foliar application of humic acid on macro and micro nutrients uptake of wheat under increasing lime levels

Lime (L), %	Humic acid treatments (HA),%							
	H_0	H_1	H_2	Means	H_0	H_1	H_2	Means
	Nitrogen, mg tdw^{-1}				Sodium, mg tdw^{-1}			
0	328.91	407.71	374.15	370.3 a	5.21 B a	8.14 A a	6.37 B a	6.57 a
5	365.76	405.31	305.55	358.9 ab	5.50 A a	5.19 A b	4.36 A b	5.02 b
10	283.90	316.25	305.80	302.0 abc	5.47 A a	4.77 A b	4.08 A b	4.77 b
20	283.16	255.37	324.72	287.7 bc	5.70 A a	4.28 A b	4.55 A b	4.84 b
40	234.14	221.65	259.40	238.4 c	4.51 A a	3.73 A b	4.15 A b	4.13 b
Means	299.2	321.3	313.9		5.28	5.22	4.70	
HA_{LSD}= ns, $L_{LSD<0.01}$= 75.017, $HAxL_{LSD}$= ns					HA_{LSD}= ns, $L_{LSD<0.01}$= 1.245, $HAxL_{LSD<0.05}$= 1.593			
	Phosphorus, mg tdw^{-1}				Iron, mg tdw^{-1}			
0	70.64 B a	91.68 A a	84.59 A a	82.31 a	1.50 AB b	1.88 A a	1.28 B a	1.56 a
5	67.62 A ab	64.43 A b	58.95 A b	63.67 b	1.14 B b	1.72 A ab	1.01 B ab	1.29 a
10	57.05 A bc	59.08 A b	53.09 A bc	56.41 b	2.00 A a	1.29 B bc	1.10 B ab	1.46 a
20	46.26 A c	38.47 A c	43.82 A c	42.85 c	1.50 A b	1.33 A bc	1.03 A ab	1.29 a
40	26.68 A d	24.42 A d	26.66 A d	25.92 d	1.03 A b	0.86 A c	0.63 A b	0.84 b
Means	53.65	56.69	53.82		1.43 A	1.42 A	1.01 B	
HA_{LSD}= ns, $L_{LSD<0.01}$= 8.529, $HAxL_{LSD<0.05}$= 10.908					$HA_{LSD<0.01}$= 0.321, $L_{LSD<0.01}$= 0.372, $HAxL_{LSD<0.05}$= 0.476			
	Potassium, mg tdw^{-1}				Copper, mg tdw^{-1}			
0	418.06	580.00	475.97	491.34 a	0.13 B a	0.20 A a	0.12 B a	0.150 a
5	445.71	401.50	388.18	411.80 ab	0.14 A a	0.10 A b	0.11 A a	0.116 b
10	416.08	407.10	342.89	388.69 b	0.12 A a	0.12 A b	0.12 A a	0.120 b
20	403.73	369.52	386.72	386.66 b	0.16 A a	0.10 B b	0.13 AB a	0.127 ab
40	295.27	267.83	264.44	275.85 c	0.12 A a	0.12 A b	0.09 A a	0.110 b
Means	395.8	405.2	371.6		0.133	0.127	0.114	
HA_{LSD}= ns, $L_{LSD<0.01}$= 83.555, $HAxL_{LSD}$= ns					HA_{LSD}= ns, $L_{LSD<0.05}$= 0.023, $HAxL_{LSD<0.01}$= 0.055			
	Calcium, mg tdw^{-1}				Zinc, mg tdw^{-1}			
0	36.62	50.06	40.17	42.28	0.25 B a	0.34 A a	0.29 AB a	0.294 a
5	40.11	37.80	33.32	37.08	0.24 A a	0.24 A bc	0.22 A b	0.232 b
10	37.97	39.27	31.58	36.28	0.24 A a	0.26 A b	0.24 A ab	0.247 b
20	39.47	35.34	38.92	37.91	0.28 A a	0.23 A bc	0.25 A ab	0.253 ab
40	37.22	30.25	36.58	34.68	0.26 A a	0.19 B c	0.26 A ab	0.237 b
Means	38.28	38.54	36.11		0.251	0.255	0.252	
HA_{LSD}=ns, L_{LSD}= ns, $HAxL_{LSD}$= ns					HA_{LSD}= ns, $L_{LSD<0.05}$= 0.023, $HAxL_{LSD<0.01}$= 0.055			
	Magnesium, mg tdw^{-1}				Manganese, mg tdw^{-1}			
0	48.44 B a	72.63 A a	52.04 B a	57.71 a	0.42	0.55	0.37	0.444 a
5	52.36 A a	53.78 A b	42.73 A ab	49.62 ab	0.37	0.40	0.31	0.363 ab
10	52.22 A a	54.92 A b	42.83 A ab	49.99 ab	0.39	0.40	0.26	0.348 b
20	53.36 A a	44.30 A bc	47.87 A ab	48.51 ab	0.36	0.28	0.28	0.308 bc
40	49.31 A a	38.02 A c	39.62 A b	42.32 b	0.28	0.22	0.28	0.260 c
Means	51.14 AB	52.73 A	45.02 B		0.364 A	0.370 A	0.300 B	
$HA_{LSD<0.01}$=6.404, $L_{LSD<0.01}$= 9.588, $HAxL_{LSD<0.05}$= 12.262					$HA_{LSD<0.01}$= 0.056, $L_{LSD<0.01}$= 0.086, $HAxL_{LSD}$= ns			

The differences between values indicated by different letters are significant. Capital letters indicate rows and small letters indicate columns
tdw: total dry weight

Discussion:

The solubility of micronutrients is particularly low and the micronutrients deficiencies were often shown on the plants grown on calcareous soils because of high pH (Kacar and Katkat, 2007). Promotion in plant growth and the nutrients uptake with the addition of humic acid had been reported by various researchers (Chen and Aviad, 1990;David, *et al.,* 1994; Fagbenro and Agboda, 1993). Kütük *et al.,* (2000). determined significant decreases on pH values of research soil (8.2 % $CaCO_3$) by the application of 2 g kg^{-1} humic acid after one-month incubation period. Obtained results are being confirmed these findings that humic acid can increase uptake of certain elements and stimulate the dry matter production of shoots. The plants take more mineral elements due to the better-developed root systems. Besides, the stimulation of ion uptake by treatments with humic materials led many investigators to propose that these materials effect to 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).

In general, according to several researches, effectiveness of the humic substances was changing relating to treatment levels, growing media and origin of humic substances. Atiyeh *et al.,* (2002) stated that beside the source of humic acid and the nature of container medium, efficiency of humic acid also differed according to the plant species. According to Lee and Bartlett (1976), application of very high dose of humic acid is less effective. Pilanalı and Kaplan (2003) were found no beneficial effects of 400 kg ha^{-1} humic acid on the growth and nutrient uptake of strawberry plant on calcareous soil (55 % $CaCO_3$). Çelik *et al.*, (2008) stated that the soil application of humus at 1 and 2 g kg^{-1} doses had statistically significant effect on dry weight, N, Na, K, Mg, Fe,Cu, Zn and Mn uptake of maize plant under calcareous soil conditions. Çimrin and Yılmaz (2005) also reported significant increases on nitrogen content of lettuce with the application of phosphorus while the application of humic acid at rates of 0-300 kg ha^{-1} did not have a significant effect. On the other hand Wang *et al.,* (1995) reported that addition of humic acids to soil with P fertilizer significantly increased the amount of water soluble phosphate, strongly retarded the formation of occluded phosphate and increased P uptake and yield by 25 %. Cooper *et al.,* (1998) applied humic acid on creeping bent grass in sand culture at rates of 100, 200, 300 mg l^{-1}, and they found that the rate of application had not effect on plant growth.

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. Fernandez *et al.,* (1996) pointed out that foliar application of leonardite extracts to young olive plants stimulated shoot growth when they were growing without the addition of mineral elements to the irrigation water, but did not promote growth when applied to plants watered with a nutrient solution, although growth of fertilized plants was greater than that of unfertilized ones. Under field conditions, foliar application of leonardite extracts stimulated shoot growth and promoted the accumulation of K, B, Mg, Ca and Fe in leaves. Govindasmy and Chandresakaran (1992) sprayed humic acid extracted from lignite to sugarcane (Saccharum officinarum L.) under the field conditions and they found that humic acid addition was improved sugar yield and nutrient concentration in leaf blades and sheaths. Tajeda and Gonzalez (2003) also reported that foliar fertilization of byproduct of olive oil mill process increased leaf carbohydrate contents and leaf N, K, Fe, Mn and Zn concentrations. Erdal *et al.,* (2000). determined the dry weight, P concentration, P uptake and residual available P amount of maize plant high in humic acid applications under 15 % lime conditions, and that the effect of humic acid on the above parameters combined with P fertilization was higher than that of humic acid alone.

Conclusion:

Humic substances may be used in case of the negative effect of calcareous soil conditions that would inhibit the plant growth and the uptake of nutrient elements. As a result of the application of humic substances under increasing lime conditions, economical levels of application should be determined and should not exceed 1 g kg^{-1} in soil and 0.1 % dose of humic acid as foliar application to non calcareous soils. Foliar application dose of humic acid must be increased to over 0.2 % under calcareous soils which have higher than 20 % of lime. Furthermore, the amount that can be applied at any one time is small and thus it requires several applications to meet the needs on calcareous soil conditions.

ACKNOWLEDGEMENT

This work was supported by the Research Fund of The University of Uludağ Project number: 2003/92 and The Scientific and Technical Research Council of Turkey (TOVAG 105 O 345).

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