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## The Effects of Sulfur and Humic Acid on Yield Components and Macronutrient Contents of Spinach (*Spinacia Oleracea* Var. *Spinoza*)

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**Abstract:** In this study, different doses of sulfur (0, 125, 250 and 375 g m<sup>-2</sup>) and humic acid (0, 10, 20 and 30 g m<sup>-2</sup>) were incorporated into soil to determine the effects of sulfur (S) and humic acid (HA) on yield components and nutrient contents in spinach. The applications of HA increased total yield of spinach. The relationship between increasing doses of S and HA with the total yield was found significant (p<0.05). But the relationships between doses of S and HA with yield components of spinach were not significant statistically. According to the results, increasing doses of S and HA increased N and P contents in plants. The relationships between HA doses and N content of plant were found significant (p>0.05). But, none of the application did affect K, Ca and Mg contents in plants statistically.

**Key words:** Sulfur, humic acid, spinach, yield, macronutrients

### INTRODUCTION

Sustainable agriculture, recently, has become popular in the world. There are several types of inorganic and organic additives used in agriculture to improve and sustain yield and yield quality of plants. Vacha *et al.*<sup>[1]</sup> studied the effects of some organic and inorganic additives on the changeover of the mobility of heavy metals in the soil and the transfer of the elements from the soil into the plants. They reported that the efficiency of the use of organic additives strongly depended on the quality of organic matter. Soil reaction has a significant affect on plant nutrient availability. The plant nutrients are usually the most available among 6.5-7.5 pH ranges<sup>[2]</sup>. Besides the essential plant nutrients, the sulphur and its compounds are commonly applied to many agricultural areas for amelioration of salty and alkaline soils<sup>[3]</sup>. When the elemental form of sulphur is applied to soils, it is transformed into the sulphate by oxidation; therefore, the soil reaction becomes more acidic<sup>[4]</sup>.

It is known that the productivity of soil is closely related to the amount of soil organic matter content. In soils, humic and fulvic acids are formed by the breakdown and decomposition of organic matters. It has been reported that these acids form strong compounds by interacting with the plant nutrients and some toxic elements in the soil<sup>[5]</sup>. Humic substances of soils play some direct and indirect important roles in the plant nutrition. Some indirect effects are the adsorption of water, the amelioration of soil by the drainage and aeration and the absorption of plant nutrients by the plant

roots. The direct effects of humic acids to plants are realized by affecting the root development and the metabolisms of plant nutrients absorbed by the plants<sup>[6]</sup>. Thenmozhi *et al.*<sup>[7]</sup> reported that the application of humic acid with fertilizer significantly influenced the growth, yield and quality parameters in groundnut. The objective of this study was to determine the effects of the various rates of inorganic and organic additives such as; sulphur and humic acid, on the yield and macronutrient contents of spinach (*Spinaceae oleracea* var. *Spinoza*).

### MATERIALS AND METHODS

This study was conducted at the Research Farm of the Horticulture Department of Yüzüncü Yıl University, Van-Turkey. Van is located between 37°55' and 39°24' north longitudes and between 42° 05' and 44° 22' east latitudes and 1720 m above sea level. Some soil physical and chemical properties of the soil were determined as follows; texture by Bouyoucos' hydrometer method<sup>[8]</sup> organic matter by the modified Walkley Black method, total nitrogen by Kjeldahl method, available phosphorous by the Olsen method, potassium, calcium and magnesium by an extraction with 1 N neutral ammonium acetate<sup>[9]</sup>, pH in 1:2.5 soil:water suspension by pH-meter, salt content of the same suspension by EC-meter, lime by Scheibler calcimeter<sup>[10]</sup>.

The certified *Spinoza* cv. was used as plant material. Four doses of humic acid (0, 10, 20 and 30 g HA m<sup>-2</sup>) and four doses of elemental sulphur (0, 125, 250 and 375 g S m<sup>-2</sup>) were arranged in forty-eight plots

according to Completely Randomized Factorial Design with three replications. The four grams of spinach seeds were sown into the plots of 1 m<sup>-2</sup> size having four rows on September 25th 2001. The constant rate of 40 kg N ha<sup>-1</sup> urea and 30 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> triple-superphosphate were applied to experimental plots with sowing. The rate of emergence was determined on October 9th 2001 and the plants were harvested on May 13th 2002. Leaf samples, oven-dried at 68°C for 72 h and ground, were digested in a mixture of nitric and perchloric acid (4:1, HNO<sub>3</sub>:HClO<sub>4</sub>) and analyzed for P spectrophotometrically and for Ca, Mg and K using atomic absorption spectrophotometer according to Kacar<sup>[11]</sup>. Total N contents in samples were determined by the Kjeldahl method<sup>[11]</sup>. The experimental data was analyzed by the SAS statistic program and significantly different means numbered according to Duncan's Multiple Range Test<sup>[12]</sup>.

**RESULTS**

According to the analysis' results, the soil had sandy-loam texture, medium in lime content, low in salt content and was slightly alkaline in pH; low in organic matter. The soil was adequate in phosphorous and magnesium contents (Table 1).

The HA applications and the interaction of the S and HA, but not sole S applications, had significant effects (p<0.05) on the yield of spinach while there were no significant effects of the S and HA applications

on the rate of emergence, the petiole length and the number of leaves (Table 2 and 3). Regardless of S application, increasing doses of HA increased the spinach yield. While the lowest mean yield (1810 g m<sup>-2</sup>) was obtained from the control treatment, the highest doses of HA (H<sub>3</sub>) gave the highest mean yield (2340 g m<sup>-2</sup>).

For the interaction of sulphur and HA applications, the S<sub>0</sub>HA<sub>3</sub> application gave the highest spinach yield (2550 g m<sup>-2</sup>) while the lowest spinach yield (1390 g m<sup>-2</sup>) was obtained from the S<sub>2</sub>HA<sub>2</sub> treatment. The S<sub>0</sub>HA<sub>3</sub> application gave the highest spinach yield (2550 g m<sup>-2</sup>) (Table 3).

The S applications had only significant effects (p<0.05) on the N and P contents of spinach while the HA applications were only significant (p<0.05) on the nitrogen content of spinach (Table 4 and 5). Moreover, the N and P contents of spinach were also significantly (p<0.05) affected by the interaction of the S and the HA applications.

Regardless of HA applications, increasing doses of S significantly increased the mean N content of spinach (p<0.05). While the lowest mean N content of spinach (0.62%) was obtained from the control application of sulphur, the highest doses of S<sub>3</sub> gave the highest mean N content of spinach (0.85%) (Table 4). Similarly, increasing doses of HA significantly increased the mean N content of spinach regardless of S applications (p<0.05). While the lowest mean N content of spinach (0.54%) was obtained from the control,

Table 1: Some physical and chemical properties of experimental soil

	pH	Salt	lime	Organic matter	Available P	Exchangeable		
						K	Ca	Mg
Texture class	(1:2.5)	(%)	(%)	(%)	(ppm)	(ppm)	(ppm)	(ppm)
Sandy-loam	8.48	0.03	6.6	0.49	13.0	21	184	256

Table 2: Effects of sulphur and HA treatments on some plant traits of spinach

Traits	Sulphur doses (g kg <sup>-1</sup> )				Humic acid doses (g kg <sup>-1</sup> )			
	0	125	250	375	0	10	20	30
Emergence (%)	17.04	18.32	18.41	16.86	18.28	16.74	18.03	17.59
Petiole length (cm)	5.69	5.13	5.06	4.81	4.93	5.32	5.31	5.13
No of leaves	7.18	7.99	8.09	7.44	7.79	7.47	7.92	7.52

Table 3: Effects of sulphur and HA interaction on the yield of spinach (g m<sup>-2</sup>).

Sulphur doses (g kg <sup>-1</sup> )	Humic acid doses (g kg <sup>-1</sup> )				Mean
	0	10	20	30	
0	1800a-d*	2510a	2310ab	2550a	2293
125	1650a-d	1900a-d	1980a-d	2500a	2008
250	2040a-d	2450a	1390d	1890a-d	1943
375	1990a-d	1465cd	2220a-c	2420a	2224
Mean	1810B**	2081AB	1975AB	2340A	

Lower case letter(s) (\*) are the interactions and upper case letter(s) (\*\*) are the means significant at p<0.05

Table 4: Effects of sulphur and HA interaction on the nitrogen content of spinach (%).

Sulphur doses (g kg <sup>-1</sup> )	Humic acid doses (g kg <sup>-1</sup> )				Mean
	0	10	20	30	
0	0.55c-e*	0.81 a-c	0.75a-d	0.40e	0.62B**
125	0.51c-e	0.37e	1.02a	1.00ab	0.72AB
250	0.67b-e	0.88ab	0.51c-e	0.93ab	0.74AB
375	0.46de	1.01a	1.01a	0.94ab	0.85A
Mean	0.54B**	0.76A	0.82A	0.81A	

Lower case letter(s) (\*) are the interaction and upper case letter(s) (\*\*) are the means significant at p<0.05

Table 5: Effects of sulphur and HA interaction on the phosphorus content of spinach (%).

Sulphur doses (g kg <sup>-1</sup> )	Humic acid doses (g kg <sup>-1</sup> )				Mean
	0	10	20	30	
0	0.33d*	0.47ab	0.36cd	0.36cd	0.40B**
125	0.43b-d	0.33d	0.46bc	0.46bc	0.41B
250	0.41b-d	0.45bc	0.40b-d	0.40b-d	0.43B
375	0.44bc	0.44bc	0.56a	0.56a	0.48A
Mean	0.40	0.42	0.45	0.45	

Lower case letters (\*) are the interaction and upper case letter (\*\*) are the means significant at p<0.05

Table 6: Effects of sulphur and HA treatments on some nutrient contents of spinach (%).

Nutrients	Sulphur doses (g kg <sup>-1</sup> )				Humic acid doses (g kg <sup>-1</sup> )			
	0	125	250	375	0	10	20	30
K	6.24	5.69	5.93	6.22	5.61	6.08	6.08	6.30
Ca	1.25	1.24	1.23	1.28	1.26	1.23	1.21	1.32
Mg	0.51	0.49	0.49	0.52	0.55	0.49	0.48	0.49

the HA<sub>2</sub> treatment gave the highest mean N content of spinach (0.82%). For the interaction of sulphur and HA applications, while the lowest N content of spinach (0.37%) was obtained from the S<sub>1</sub>HA<sub>1</sub>, the S<sub>1</sub>HA<sub>2</sub> application gave the highest nitrogen content of spinach (1.02%).

Increasing doses of S significantly increased the mean P content of spinach regardless of HA applications (p<0.05). While the lowest P content of spinach (0.40%) was obtained in the control, the highest doses of S<sub>3</sub> gave the highest mean P content of spinach (0.48%) (Table 5). Similarly, increasing doses of HA increased the mean P contents of spinach; however, these increases were not significant statistically. While the lowest mean P content of spinach (0.40%) was obtained from the control application, the HA<sub>3</sub> and HA<sub>2</sub> treatments gave the highest mean P contents of spinach (0.82%). For the interaction of S and HA applications, while the lowest P content (0.33%) was obtained in the S<sub>0</sub>HA<sub>0</sub>, the S<sub>3</sub>HA<sub>2</sub> application gave the highest P content in spinach (0.56%).

Effects of S and HA applications on K, Ca and Mg contents of spinach are given in Table 6. Changes in these macro nutrient contents of spinach by the application of S and Ha were not significant statistically.

### DISCUSSION

According to the results of the study, although there was no significant effect of the S and HA applications on the rate of emergence, the petiole length and the number of

leaves, increasing doses of HA increased the mean yield of spinach up to 27%. Studies such as Senesi *et al.*<sup>[13]</sup>, Wang *et al.*<sup>[14]</sup>, also reported that HA applications increased the crop yield. Lobartini *et al.*<sup>[6]</sup>, stated that the ameliorative effects of HA on the plant yield might have been come from the effects of HA on the adsorption of water and the physical structure of soil by the drainage and aeration and the absorption of plant nutrients by the positively affected plant roots by HA and the metabolisms of plant nutrients absorbed by the plants.

Increases in the mean N contents of plants in the present study were 22 and 51% higher in the S<sub>3</sub> and HA<sub>3</sub> treatments over the control treatment, respectively. The increases by S are in agreement with the findings of several other researchers<sup>[15,16]</sup>. It was determined that HA was more effective in the N increment than S. Fagberno and Agboola<sup>[17]</sup>, David<sup>[18]</sup>, Adani<sup>[19]</sup> also reported that HA applications increased the N contents of plants.

Compared with the control treatment, S<sub>3</sub> treatments increased the mean P contents of plants by 18%. This is in line with the results of Ashby *et al.*<sup>[20]</sup> and Erdal *et al.*<sup>[21]</sup>. Although HA applications increased the mean P content of plants up to 18%, this was not statistically significant.

In the present study, no significant difference was detected in the K, Ca and Mg contents of plants by the S and HA applications. The relatively lower doses of these substances might have caused those insignificant increases.

Besides being a source of N itself, HA became more effective than S in the yield and the N content of spinach because of its high cation exchange capacity and chelating effect<sup>[22]</sup>. Although sulphurous fertilizers positively affect the crop development, it has been reported that there have been significant decreases in pH and organic carbon of soil, the massive growth of bacteria and the rate of C/N; increases in the total S and SO<sub>4</sub><sup>-2</sup> concentrations due the oxidation of elementary S<sup>[23]</sup>. It has been thought that S applications did not significantly affect the spinach yield based on the literature knowledge and the very low organic matter content of soil in the experimental area.

The general aims of the studies in the fields of soil nutrition and soil fertility are to increase the crop yield and quality. Thus, it is known that the applications of synthetic fertilizers become common recently. On the other hand, it is necessary to avoid excessive applications of fertilizer for both economic and environmental reasons. Therefore, it might be useful to apply HA whose positive effects on the uptake of plant nutrients and the crop yield. More detailed studies conducted on soils with different fertility levels are needed to clarify the effects of different doses of S on the yield and nutrient uptakes of various crops.

## REFERENCES

1. Vacha, R., E. Podlesakova, J. Nemecek and O. Polacek, 2002. Immobilisation of As, Cd, Pb and Zn in agricultural soils by the use of organic and inorganic additives. *Rostlinna Vyroba*, 48: 335-342.
2. FAO, 1984. Guidelines: Land evaluation for rainfed agriculture. FAO soils Bulletin No. 52, FAO Rome, pp: 335.
3. Tisdale, S.L., B.R. Bertramson, 1959. Elemental sulfur and its relationship to manganese availability. *Soil Sci. Soc. Am. Proc.*, 17: 131-137.
4. Bohn, H.L., B.L. McNeal and G.A. O' Connor, 1985. Soil chemistry. 2 Edn., John Willey and Sons Inc., pp: 324-325.
5. Harper, S.M., G.L. Kerven, D.G. Edwards and Z. Ostatek-boczyski, 2000. Characterization on fulvic and humic acids from leaves of eucalyptus comaldulensis and from decomposed hay. *Soil Biol. Biochem.*, 32: 1331-1336.
6. Lobartini, J.C., G.A. Orioli and K.H. Tan, 1997. Characteristics of soil humic acid fractions separated by ultrafiltration. *Com. Soil sci. Plant Anal.*, 28: 787-796.
7. Thenmozhi, S., S. Natarajan and G. Selvakumari, 2004. Effect of humic acid on growth and yield parameters of groundnut (var. Vri 2). *Cropres.* 27: 205-209. Dept. Soil Sci. Agric. Chem. Tamil Nadu Agric. Univ., Coimbatore-641 003 (Tamil Nadu), India.
8. Bouyoucos, G.L., 1951. A recalibration of the hydrometer for making mechanical analyses of soil. *Agron. J.*, 43: 434-437.
9. Kacar, B., 1994. Chemical analysis of plant and Soil Soil Analyses. Ankara Univ. Fac. Agric. No. 3, Ankara, Turkey.
10. Jackson, M.L., 1962. Soil Chemical Analysis. Prentice Hall. Inc. Eng Cliffs. New Jersey.
11. Kacar, B., 1984. Plant Nutrition Application Handbook. Ankara Univ. Fac. Agric. No. 900, Lab. Practice guide no:214. Ankara.
12. SAS Inst., 1988. PC SAS User's Guid: Statistics. SAS Inst. Inc. Cary, NC.
13. Senesi, N., E. Loffredo and G. Padonava, 1990. Effects of humic acid. Herbicide interactions on the growth of pisum sativum in nutrient solution. *Plant and Soil*, 127: 41-47.
14. Wang, C.D., H.T. Chan and C.L. Lay, 1991. Effects of organic manures on the yield and quality of grapes. *Bulletin of Taichung District Agricultural Improvement Station*, pp: 41-48.
15. Cadwell, A., E. Seim and G.W. Rehm, 1969. Sulfur effects on the elemental composition of alfalfa (*medicago sativa* l.) and corn (*zea mays* L.). *Agron. J.*, 61: 632-634.
16. Soliman, M.F., S.F. Kostandi and M.L. Beusichem-Van, 1992. Influence of sulfur and nitrogen fertilizer on the uptake of iron, manganese and zinc by corn plants grown in calcareous soil comm. *Soil Sci. Plant Anal.*, 23: 1289-1300.
17. Fagberno, J.A. and A.A. Agbolla, 1993. Effecth of different levels of humic acid on growth and nutrient uptake of treak seedling. *J. Plant Nutr.*, 16: 1465-1483.
18. David, P.P., 1991. Effects of applied humic acid on yield, growth, nutrient accumulation/content in selected vegetable crops and soil. Interaction that reduce their effectiveness. *Dissertation abstracts- international*, 52: 1136-1137.
19. Adani, F., P. Genevi and G. Zocchi, 1998. The effect of commercial humic acid on tomato plant growth and mineral nutrition. *J. Plant Nutr.*, 21: 561-575.
20. Ashby, D.L., W.E. Fenster and O.J. Attoe, 1966. Effect of partial acidulation and elemental sulfur on avability of phosphorus in rock phosphate. *Agron. J.*, 58: 621-625.
21. Erdal, I., M.A. Bozkurt and K.M. Cimrin, 2000. Effects of humic acid and phosphorus applications on corn (*Zea mays* L.) growth and phosphorus uptake in a calcareous soil. *Turk J. Agric. For.*, 24: 663-668.
22. Senn, T.L. and A.R. Kingman, 1973. A review of humus and humic acids. Clemson university and determent of horticulture, research series, March, No. 145.
23. Alexander, M., 1977. Inroduction to Soil Microbiology. 2 Edn. John Willey and Sons Inc, pp: 350-368.