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Sulfur application effect on pH measurement of wheat rhizosphere in calcareous soils

Shafagh Vali Shariat Panahi*, Farhad Moshiri** and Mohammad Reza Ardakani*
**Department of Agronomy, Karaj Branch, Islamic Azad University, Karaj, Iran*
***Water and Soil Research Institute, Karaj, Iran*

(Corresponding author: Farhad Moshiri)
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ABSTRACT: The experiment was carried out in factorial form and unbalanced randomized complete block design with three replications under greenhouse conditions at Soil and Water Research Institute. The variables included three types of soil sample (Sarvestan district (Fars Province), Darin Mianeh (East Azerbaijan Province) and Esfarayen desert (North Khorasan Province)) and sulfur levels (0, 1000 and 5000 kg/ha). Each soil sample was treated with different proportions of thiobacillus bacteria and the seeds of Alvand cultivar (the best cultivar of wheat) were planted in each rhizobox (Root Box). The results indicated that sulfur application had significant effect not only on adjusting the amount of soil pH ($P<0.01$) but also on the amount of sulfate and shoot dry weight ($\alpha \leq 0.05$). The maximum shoot dry weight and the minimum root dry weight of the plant belonged to the soil of Esferayen desert. By sulfur increasing, no significant change was observed in the shoot sulfur amount in the soil of Darin Mianeh.

Keywords: oxidation, wheat cultivar, soil acidity, sulfur, rhizosphere

INTRODUCTION

In most parts of Iran, the calcareous parent materials and low precipitation leads to the formation of alkaline and calcareous soils. Soil pH is considered as the most important element of the soil in determining the absorbency of nutrients that exist in the soil for plants (Wang *et al*., 2001). In soils with high pH, phosphorus and potassium react with calcium and become insoluble, and thus become unavailable to the plant roots (Hopkins and Ellsworth, 2005; Mostashari *et al*., 2008). The oxidation depends on various factors including; temperature, moisture, pore size and sulfur particles of soil (Siami *et al*., 2008). Manure, as one of the best alternatives to chemical fertilizers, improves physical, chemical and biological properties of eroded and low productive soil (Eghbal *et al*., 2004). Within the rhizosphere, microorganisms positively affect plant health through a variety of mechanisms, including mineralization of nutrients, suppression of disease, improving plant stress tolerance, and production of phytohormones (Berendsen *et al*., 2012; Figueiredo *et al*., 2011; Gupta *et al*., 2000). In a study by Kayser *et al*. (2000), the application of elemental sulfur and a decrease in soil pH increased the solubility of heavy metals. Today, It has been shown that sulfur is an influential factor to enhance and adjust the pH of calcareous soils. In the presence of thiobacillus as a sulfur oxidizing bacteria, elemental sulfur is converted to sulfuric acid which can reduce the soil pH and increase the accessibility of nutrients such as phosphorus. Sulfuric acid composed from the oxidation of sulfur and changes the insoluble phosphate compounds to soluble. The sulfur existed in some fertilizers and amendments are in the form of rebirth; whereas, the available sulfur in plants and many microorganisms is mostly in the form of oxidized sulfur. On the other hand, adding sulfur to soil enhances the nutritional status of plants through the release of nutrients such as phosphorous, zinc, copper, iron and etc. (Malakouti and Riazi Hamedani, 1991). In a study, it was indicated that the rate of sulfur oxidation in soils inoculated by thiobacillus bacteria was about 11 times more than non-inoculated soils (Besharati Kelayeh, 1998).

In the present research it was assumed that the process of sulfur oxidation increases in plant rhizosphere, due to certain chemical circumstances and more microbial activity in compared with soil mass. Therefore, the current research was conducted to investigate the effect of sulfur consumption and thiobacillus bacteria on the oxidation of wheat sulfur in calcareous soils under greenhouse and laboratory conditions.

MATERIAL AND METHODS

The experiment was carried out in factorial form and unbalanced randomized complete block design with three replications under greenhouse and laboratory conditions at Soil and Water Research Institute.

A. Soil samples preparation

12 soil samples were initially analyzed and with regard to the results, 3 samples (Table 1) with different pH close to alkaline were selected (Darin Mianeh district in East Azerbaijan (7.9 pH), Esferayen desert (7.96 pH) and Sarvestan district in Fars (8 pH). The other variable were sulfur consumption in three levels (0, 1000 and 5000 kg/ha). First, the weight of 1 hectare of soil samples was calculated in depth of 3 cm and based on the obtained values, sufficient amount of pulverized sulfur powder was added to the each of rhizobox. The applied treatment to each 3 kg soil sample was composed of sulfur treatment + powdery thiobacillus inoculants (as much as 4 wt% of consumed sulfur in each treatment) + 12 g of Vermicompost. Afterwards, the soil moisture content reached to field capacity (FC) by distilled water and was laid down under greenhouse conditions for one month.

Table 1: Physicochemical properties of the examined soil.

Soil Type	P (AV.) ppm	O.C	T.N.V	CEC
Sarvestan	2	0.3	21.7	10.8
Darin Mianeh	4	0.61	10.3	28.6
Esfarayen Desert	7.6	0.22	33.1	10.6

B. Seed cultivation and pH measurement

After a month, the stages of soil samples laying and seeds cultivation in rhizoboxes where sequentially followed in order to measure the rate of plant growth and pH changes in rhizosphere of the plant (Wenzel *et al*., 2001). Rhizoboxes were filled with treated soil until they reach to the bulk density of 1.4 g/m^3 and then a number of 20 wheat seeds were planted in it. Two weeks after cultivation, 10 plants remained under equal circumstances. 8 weeks later, when dense root plates were produced, the plants were cut from the crown and rinsed with tap and distilled water. Then prepared samples were dried at 65°C in the oven. Subsequently, the amount of pH, sulfate, dissolved organic carbon and shoot were measured. The available sulfur was measured through turbidity method using a Spectrophotometer (Singh *et al*., 1984).

C. Statistical analysis

Data were analyzed by SAS software. The comparison of the Means was done based on Duncan's Multiple Range Test (DMRT) at %5 probability level.

RESULTS AND DISCUSSION

The results of the variance analysis (Table 2) showed that there was statistically significant difference in shoot dry weight ($P<0.01$) and root dry weight ($P<0.05$) between different types of soil. Nonetheless, interaction effect of sulfur and soil type had no significant effect on shoot sulfur; whereas, it had a statistically significant difference on root and shoot dry weight ($P<0.05$).

Table 1: The Variance Analysis of soil type and sulphur effect on the properties of soil and plant.

SOV	df	Mean Squares		
		Shoot dry weight	Root dry weight	Shoot sulfur
Sulfur	2	0.24 ns	0.07 ns	82.68 ns
Type of Soil	2	3.65 **	0.15 *	18.51 ns
Sulfur*Type of Soil	4	0.41 *	0.17 *	50.09 ns
CV (%)		10.67	26.80	41.20

ns, * and ** indicate no significant difference, significant difference at 5 and 1%, respectively

The comparisons showed that sulfur consumption did not have any significant effect on root dry weight and shoot sulfur. 5000 kg/ha of sulfur consumption significantly increased shoot dry weight (Table 3). In addition, means comparisons of diverse soils (Table 4) showed that the highest shoot dry weight of wheat belonged to the soil of Esfarayen district; On the contrary, this soil had the lowest root dry weight. It seems that there was a reverse relationship between root and shoot dry weight. It is probable that sulfur had increased the accessibility of nutrients such as phosphorous by reducing soil pH and nutrients transferring across the roots had resolved the shoots need or more dry matter had been used up by the shoot.

Table 3: The Mean Comparison of sulfur application on the upper part of the rhizobox (root box) at measured characteristics.

Sulfur treatment	Shoot Dry Weight (g/pot)	Root Dry Weight (g/pot)	Shoot sulfur (mg/kg)
0	2.73b	0.85a	22a
1000	2.49b	0.72a	28.50a
5000	4.60a	0.67a	24.12a

Similar letters in each column indicate no significant difference between the means.

Table 4: The Mean Comparison of the soil type effect on wheat at measured characteristics.

Soil Type	Shoot dry weight (g/pot)	Root dry weight (g/pot)	Shoot sulphur mg/kg
Darin Mianeh (East Azerbaijan)	2.35b	0.71a	22.75a
Sarvestan (Fars)	2.31b	0.91a	26.88a
Esfarayen (North Khorasan)	3.58a	0.61b	24.37a

Similar letters in each column indicate no significant difference between the means.

In spite of the fact that no significant difference was statistically observed in the of shoot sulfur amount between the different types of soil but the highest shoot sulfur amount (26.88 mg/kg) mathematically belonged to the Sarvestan (Fars) soil while the lowest amount (2 mg/kg) belonged to Darin Mianeh (Table 4). Regarding to the results presented in Table 3, sulfur consumption effect on the measured traits showed that 0 and 1000 kg/ha of sulphur consumption did not affect shoot dry weight; while, 5000 kg/ha treatment had two more times effect on it. After 3 years of experimentation on the calcareous soil under cultivation of vineyards, Goudarzi (2007) reported that the application of sulfur with thiobacillus reduced the soil pH. By applying 0.5 kg sulfur per plant, he observed that the soil pH dropped on average by 0.56 from 7.23 to 6.67 units after 3 years. These findings showed that the application of sulphur did not pose any significant changes on root dry weight; that is while increasing sulfur amount not significantly change/increase shoot sulfur in comparison to control treatment (Table 3). This would be probably due to the accumulation of phosphorous in rhizosphere to maximize the rate of absorbency than the rate of spread and increase the growth rate of shoot than root.

The means comparison results of sulfur application on Darin Mianeh soil showed that no significant increase was observed on shoot dry weight, root dry weight and shoot sulfur by increasing sulphur amount (Table 5).

In addition, although increasing the amount of consumed sulfur did not show any statistically significant changes on the amount of shoot sulfur in comparison to the control group, it was still high along with its values in all the treatments. It seems that increasing sulfur application up to 1000 kg/ha showed no more changes on the amount of shoot sulphur. Thus, the absorption threshold was fixed. Therefore, by increasing the amount of sulfur application from 1000 up to 5000 kg/ha, the root and shoot dry weight was also fixed and did not significantly change. The results revealed (Table 5) that by sulfur increasing at its various levels, shoot dry weight in the soil of Sarvestan (Fars) was still the highest value; whereas, the root dry weight decreased by increasing its amount. On the other hand, shoot sulfur increased at 1000 kg/ha of sulfur level in comparison to its non-application, while decreased at the third level of sulfur. According to the results presented in Table 5, it was realized that 0 and 1000 kg/ha of sulphur application did not show any significant changes on shoot dry weight; whereas, increasing sulfur level to 5000 kg/ha subsequently increased the absorbency about 1 gram per rhizobox. Furthermore, the findings indicated that by increasing the amount of sulfur, shoot dry weight increased as well. However, there was no statistically significant difference between the application of sulfur at its various levels and the amount of shoot sulphur, and the values of shoot sulfur still remained high, even though the amount of sulfur got increased to its various levels.

Table 5: The mean comparison of sulfur application effect on the soil samples at measured characteristics.

Soil sampels	Sulfur treatment	Shoot dry weight (g/pot)	Root dry weight (g/pot)	Shoot sulfur (mg/kg)
Darin Mianeh (East Azerbaijan)	0	2.166a	0.807a	18.33a
	1000	2.393a	0.575a	25.33a
	5000	2.513a	0.775a	25.50a
Sarvestan (Fars)	0	2.70a	1.28a	23.33b
	1000	2.11a	0.80b	34.67a
	5000	2.13a	0.65b	22.67b
Esfarayen (North Khorasan)	0	3.33 b	0.47 b	24.33 a
	1000	3.22 b	0.83 a	24 a
	5000	4.08 a	0.62 ab	24.66 a

Similar letters in each column indicate no significant difference between the means.

According to the results of the research, Table 1 displayed that the degree of soil acidity is effective on the growth rate of plants. Based on Table 2, shoot growth rate was higher in alkaline soil than the other two soil samples; thus, it seems that sulfur is more effective on alkaline soils because sulfur impeded from nutrients absorption, including micronutrients, across the plants in calcareous soil with alkaline pH; hence, it caused problems in plants growth and development (Wang *et al.*, 2001).

However, this conclusion was not confirmed by further review of Table 2 since not any statistically significant difference was observed in the amount of shoot sulfur. Consequently, it is worth to pay more attention to the traits of the shoot. one of the interesting results was decrease in shoot sulfur of Esfarayen soil which indicating photoassimilates allocation to different shoots and organs of the plants. More, if shoot is aimed at plant harvesting, many factors should be taken into account in terms of crop management including the cultivation date/time, irrigation and nutrition of plants so that less root will be produced because a plant can photosynthesize up to a certain degree; therefore, if more roots be produced, they certainly need more photoassimilates to be allocated to the plant which, in turn, decrease the plant's shoots yield (Nourmohammadi *et al*, 2001). As a consequence, it seems that the high performance of shoots in the soil of Esfarayen was due to the limitation caused by existence of alkaline in the same soil for root growth as well as less photoassimilates allocated to the production and growth of the plant's root. If fewer roots be produced, with the passage of time and in case of lengthier experimentation period, its effects will be more likely to be noticed since the CO2 produced from the respiration of roots and microorganisms has been reported as one of the most influential factors on the changes of pH (Hinsinger, 1999; Marschner and Romheld, 1996).

Table 3 illustrated that there was not any certain trend in either the decrease or increase of absorption due to various reasons including the proximity of the pH of the soils, short period of experimentation, the amount and type of consumed sulfur and other factors. The other factors that play a crucial role in the changes of soil pH amount include not only the initial pH of the soil measured in the beginning of the research (Nye, 1986) but also the secretion of proton, hydroxyl ion and bicarbonate to neutralize the charges caused by the additional absorption of cations and anions (Jaillard *et al*., 1995; Hinsinger *et al*., 2003), as well as the secretion of organic acids and amino acids by roots and microorganisms (Hinsinger *et al*., 2003; Marschner, 1995), the processes of oxidation and reduction (Cifuentes and Lindeman, 1995) and -5 and -6 buffering capacity of the pH of soils (Hinsinger *et al*., 2003), all of which requires to be further investigated in future studies.

The results of Table 5 illustrated the role of calcareousness and alkalinity of the soil as well as the effect of sulfur. There was no significant difference in the measured traits of Darin Mianeh; thus, one of the main reasons contributing to the lack of significant difference was the low alkalinity of soil. On the contrary, it was indicated that the root (the Sarvestan soil), and the root and shoot (Esfarayen) had been significantly changed.

Nevertheless, it is worth to review the research results of the other researchers and compare their findings with the results of the present research hereunder.

After 3 years of experimentation on the calcareous soil under cultivation of vineyards, Goudarzi (2007) reported that the application of sulfur with thiobacillus reduced the soil pH.

Malakouti (1991) stated that sulfur was effective provide and oxidized to a considerable amount which was possible when sulfur oxidizing bacteria exist sufficiently in the soil especially in various genus of thiobacillus. According to Malakouti and Rezaei (2001), the application of sulphur was effective in calcareous soil. They stated that annual application of sulfur along with manure could decrease the soil pH from 8.2 to 7.8 and increase the solubility of phosphorous after 5 years. However, in case of using sulfur dissolvent, this pH decrease can occur within 3 years. Moafpourian *et al* (2007) found that by increasing the amount of absorbable sulfur of the soil (extractable with mono-calcium phosphate), the soil pH decreased as well. More than %70 of the changes in the pH of these soils was dependent on the amount of absorbable sulfur. It can be concluded that research on the application of sulfur and pH changes is time consuming. In order to achieve better results, it would be better to design and conduct long-term experimentations. As a final remark, the most important findings of the present research was that long-term research and experimentations are needed to accurately observe the changes of soil pH and sulfur effects on these changes.

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