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Effect of *Thiobacillus* bacteria, sulfur and manure on the nutrient and pH of soil in garlic (*Allium sativum*)

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Abstract

This study was conducted as split plot factorial based on randomized complete block design in 3 replicates and 18 treatments were carried out during 2011-2012 at Agronomy and Plant Breeding Department, College of Agriculture and Natural Resources, Razi University, Kermanshah, Iran. In this experiment the main plots consisted of a factorial combination of manure (0, 10 and 20 tons.ha^{-1}) and sulfur (0, 250 and 500 kg.ha^{-1}) and subplots includes *Thiobacillus* bacteria (no use and use), respectively. The result of the experiment revealed that application of manure at various levels with recommended dose of *Thiobacillus* bacteria enhanced the growth and yield attributes in garlic over control. Analysis of soil nutrients using animal manure in organic carbon showed significant difference for traits including available nitrogen, phosphorus, potassium, manganese and zinc. In the all of parameters mentioned in 20 tons of manure treatment and treatments of sulfur, phosphorus, potassium, manganese, iron and zinc in soil pH caused significant difference that in all aforementioned traits other than manganese treatment 500 kg of sulfur has the amount of highest. However, we find out that the presence of *Thiobacillus*bacteria reduced soil pH and rate of iron absorbed is increased. The interaction between bacteria and sulfur, zinc and pH effects on the triple interaction was significant difference at the five percent level.

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Introduction

After nitrogen, phosphorus and potassium, sulfur is considered as fourth major element in most crops (Hitsuda*et al.*, 2005), this element is present in the structure of amino acids exist despite the fact that there is no sulfur compound leaf chlorophyll However, it is essential for chlorophyll biosynthesis (Messick and Fan, 1999). Iran as an arid and semi-arid part of the world so optimum efficiency production in calcareous soils and soils with high pH, has always faced challenges. An important part of this problem as it arises in these soils due to the high pH and high concentrations of calcium ions, nutrients that are absorbed into the composition of the pH-dependent and useless for insoluble driven thereof. However, adding these element to the soil as fertilizers cause environmental pollution problems (Sameni and Kasaraian, 2004). Many researchers reported that the oxidation of sulfur and sulfuric acid production cause reducing the acidity of the soil, increasing supply needed sulfate for plants, phosphorus and micro-nutrients in the soil (Dawood*et al.*, 1985). Rate of sulfur oxidation in soils vary and depend on population Thiobacillus bacteria in soil, particle size and environmental conditions. With increasing soil sulfur, and sulfur oxidation bacteria population will large and it requires more nutrients to bacteria. Therefore, oxidation of sulfur in soils can be productive more quickly (Agrifacts, 2003). A Thiobacillus bacterium is belong to Gram-negative bacterium and provides its energy needs through the oxidation of inorganic sulfur compounds (Donat*et al.*, 1997). It is acidophilic bacteria and catalyzed iron sulfide oxidation to ferric sulfide or sulfuric acid (Gomez *et al.*, 2000).

Weather features and unsuitable management in arid and semi-arid lands has led to the loss of organic matter. This causes negative effect on biological characteristics, soil physical and chemical, destruction of soil building and loss of soil fertility (Madrid *et al.*, 2007). Today, biological organic matter and fertilizer are taken into account as an alternative to the overuse of chemical fertilizers to enhance soil fertility, especially in discussions of sustainable agriculture (Wu *et al.*, 2005). Nutrient supplies needed for plants using organic fertilizers has key role in the maintenance of soil fertility and crop production (Brouwer*et al.*, 1985) and can be improved soil physical and chemical properties. Application of manure improves eroded and inefficient soil (Eghbal*et al.*, 2004) Application of manure to improve the physical, chemical and biological properties of soil and also increase yield of crop (Kuepper, 2000).

Garlic has second major rank in the family after the onions based on importance and cultivation scale (Gomez *et al.*, 2000) and has many medicinal uses (Sanderman, 1995)..The present study aims to determine the Effect of Thiobacillus bacteria, sulfur and manure on the nutrient and pH of soil in Garlic (Allium sativum).Hereby,Maximum potential of soil has been used. Also the indiscriminate use of chemical fertilizers can be avoided. Due to the lack of reports about these factors and the impact of these factors to plant garlic in this area, this study was designed to determine the effect of organic and biological fertilizers on soil fertility, solubility of nutrient and decreasing soil pH.

Material and method

Soil properties

The experiment was conducted in Agronomy and Plant Breeding Department, College of Agriculture and Natural Resources, Razi University, Kermanshah, Iran (Latitude: 33° 36'N, longitude: 45° 24' E, and altitude 1340 m above sea level). Table 1 shows the meteorological information of the experiment location. Before attempting to cultivate a soil sample was prepared from depths were 0 to 30 cm soil physical and chemical properties of soil in the project area (Table 2).

Treatments

In this study the main plots consisted of a factorial combination of the manure (at three levels, 10 and 20 tons per hectare) and sulfur (zero, 250 and 500 kg per ha) and subplots includes *Thiobacillus* bacteria (the bacteria and without bacteria),

respectively. Operations of the land preparation, plowing, leveling and plot layout was carried out in early October and subsequent treatments including cow manure, sulfur bacterium Bacillus concurrently with each other to thirty centimeters in depth with the territory of the Project Site were mixed.

Result and discussion

Soil pH

Based on the our results, sulfur and bacteria application had significant effect on soil pH at the one and five percent level, respectively, and the triple interaction effects were significant difference at the five percent level (Table 3). Sulfur application with an average consumption of 500 kg per hectare showed lowest pH treatments, 7.69, compared to 250 kg with an average 7.37 and without sulfur with an average of 7.81, in other words by increasing sulfur the pH of soil was reduced (table 2). *Thiobacillus*bacteria application with an average of 7.37 showed lower pH compared to the absence of bacteria with an average of 7.76 pH less were followed (Table 4). According to the interaction effects, combining the use of bacteria in the presence Sulfur content and manure on soil pH will grow significantly at the five percent level (Figure 1).

Table 1. Location of meteorological conditions Experiment separately cultivated in 2011-12 year.

Parameter	sep-oct	oct-nov	nov-dec	dec-jan	jan-feb	feb-mar	mar-apr	apr-may	may-jun	jun-jul	jul-agst	agst-sep
precipitation	0	0	0	0	25.2	35.4	34.3	68.2	10.4	0.5	131	0
Humidity	284.6	367.8	361.2	304.6	120.5	82.2	-	-	-	0.2	57.5	205.3
Avg. Temp.	25.5	29	27	23.9	17.8	11.9	4.4	3	4.4	3.1	10.6	18.7
Max. Temp.	36	39	36.9	33.7	26.5	19.2	22	14.9	15.8	18.8	24.6	32.4
Min. Temp	15	19	17	14.2	9.3	4.7	-11.2	-11.2	-9	-8.7	-1.9	2.5

Mahler and Maples (1986) showed that the use of elemental sulfur has a significant role in reducing soil pH and soil pH decreased more rapidly with increasing sulfur. *Thiobacillus* bacteria by oxidation of sulfur produced some to sulfuric acid and at low buffered properties can considerably reduce pH. The intensity of biological oxidation of sulfur (S) to SO_4 in soils of is depend on population *Thiobacillus* bacteria in soil, particle size and environmental conditions. With increasing soil sulfur, sulfur oxidation and bacteria population will be increased and it requires more nutrients to bacteria. Therefore, oxidation of sulfur in the fertile soil more quickly has done (Agrifacts, 2003). Hence, manure can improve the living conditions of the soil, providing an ideal environment for bacterial activity and may play an important role in reducing soil pH. However, the amount of organic acids produced during the decomposition of organic material, which can reduce the pH.

Table 2. Physical and chemical properties of soil in the project area (0 to 30 cm depth).

Sand (%)	silt (%)	clay (%)	PH	Organic Matter (%)	Total Nit. (%)	P (mgkg-1)	K (mgkg-1)	Mn (mgkg-1)	Fe (mgkg-1)	Zn (mgkg-1)	Cu (mgkg-1)
24	41	35	7.8	1.68	0.16	16.8	370.7	7.6	6.37	0.97	2.36

Organic Carbon

Results showed significant effect of manure on the amount of soil organic carbon (0.01> P). Meanwhile, the other factors studied and their interactions had been no significant effect on it (Table 3). Between levels of manure , cow manure, with an average of 20 tons per hectare, with the average of 1.79 %, represented maximum amount of soil organic carbon were followed while consuming 10 tons of manure per hectare and no treatment, by 1.69% and 1.62% , respectively (table 4). The results Khan and Schnitzer, (1972) corresponded well with the findings of this study showed that during a test of organic fertilizers, soil organic carbon increased significantly.

Soil available nitrogen

Results of data analysis showed that the treatment combination of manure on soil nitrogen has a

significant effect (0.01> P), and no significant difference was observed in the other treatment combination (Table 3). Consumption of 20 tons per hectare of manure with the average of 18% had highest nitrogen in soil and application of 10 tons and no manure treatment with the average of 17% and 16%, respectively (Table 4). MohammadzadeNouri and Nemati (2010) also found similar results, their results showed a significant effect between the uses of manure on the available nitrogen in the soil. Pratt *et al.*, (1976) conducted a field experiment and showed that manure with 1.6 to 2.2 percent nitrogen, 40 to 50 percent during the first year, 10 to 20 percent in the second year and to 5% in third year turned to inorganic form.

Table 3. Analysis of variance of soil pH and nutrient concentrations of experimental treatments (mean square).

SOV	df	Organic Carbon	N	P	K	Mn	Fe	Zn	Cu	PH
Rep.	2	0.009	0.00008	63.04	24188	5.21	0.09	0.31	0.01	0.006*
Manure	2	0.012**	0.0015**	659.78**	162035**	16.12**	0.27	3.869**	0.08	0.0001
Solfur	2	0.0016	0.00007	1184.98**	69420**	204.76**	1.92**	22.82	0.014	0.069**
Man. * Sol.	4	0.0084	0.00006	19.73	13138	0.793	0.16	0.52	0.05	0.0004
Ea	16	0.0099	0.00007	21.52	7530	2.48	0.1	0.3	0.06	0.0014
Thiobacillus bacteria	1	0.0458	0.00006	13.08	7143	3.81	0.62*	0.68	0.05	0.011*
Thi * Man.	2	0.0228	0.00006	17.85	28160	4.14	0.16	0.02	0.01	0.0008
Thi. * Sol.	2	0.0122	0.0001	79.3	7731	3.6	0.05	0.84*	0.1	0.0004
Triple interaction	4	0.0149	0.00007	59.44	6578	3.74	0.06	0.49*	0.06	0.0058*
Error	18	0.0126	0.00005	23.05	8760	3.42	0.1	0.16	0.053	0.0019

ns, ** and * respectively non-significant, significant at 1% and 5% probability level.

Soil available phosphorus

Our results also showed highly significant effect of manure and sulfur application on soil available phosphorus (0.01> P) (Table 3). The 20 tons of manure per hectare, with an average 35.29 mg/kg had the highest rate of soil P and treatments 10 tons per hectare and without manure, showed average of 29.64 and 23.15 mg, respectively (Table 3). So that the amount of phosphorus in the soil by increasing the amount of manure increased from zero to 20 tons per hectare. The consumption of 500 kg per hectare of sulfur with an average of 37.78 mg was maximum amount of available phosphorus in soil against the treatment of 250 kg sulfur and without sulfur with an average of 28.62 and 21.59, respectively (table 4).

Table 4. Mean comparison of soil pH and nutrient concentrations of experimental treatments.

Treats		Organic Carbon	N	P	K	Mn	Fe	Zn	Cu	PH
Manure	No fertilizer	1.62B	0.16c	23.15c	395.33c	10.44a	6.58a	1.92c	2.4a	7.74a
	10 ton per hectare	1.69b	0.17b	29.64b	466.27b	10.89b	6.55a	2.38b	2.4a	7.75a
	20 ton per hectare	1.79a	0.18a	35.29a	583.22a	12.26a	6.78a	2.85a	2.52a	7.74a
Sulfur	No sulfur	1.69a	0.17a	21.59c	427.56c	8.04c	6.28b	1.13c	2.38a	7.81a
	250 kg per hectare	1.71a	0.17a	28.62b	467.83b	14.75a	6.71a	2.72b	2.4a	7.73b
	500 Kg per hectare	1.69a	0.17a	37.78a	549.44a	10.8b	6.92a	3.31a	2.55a	7.62c
Thiobacilous	No Bacteria	1.67a	0.17a	29.84a	493.11a	10.93a	6.53b	2.27a	2.41a	7.76a
Bacteria	With Bacteria	1.73a	0.17a	28.85a	470.11a	11.46a	6.74a	2.5a	2.47a	7.73b

The amount of available phosphorus in soils has been confirmed in additional research, for example, Eghbal and Power (1999) reported the application of manure, increased the amount of phosphorus in the soil. On the other hand, Kwabiah*et al.*, (2003) have also expressed organic matters and their composition reduces soil phosphorus fixation is compared with inorganic phosphate. Kaplan and Oram (1988) showed that in calcareous soil acidity, 8.7 after the addition of various amounts of sulfur had significant effect on pH reduction and increasing P uptake. Eghbal and Power (1999) positively evaluated the

effect of sulfur on the release of phosphorus from rock phosphate, and increasing yield. In calcareous soils of arid and semiarid climates in Iran, high pH, high percentage of calcium carbonate, little organic matter and soil drought has caused a significant amount of phosphorus is less than the amount required for optimum growth of most crops.

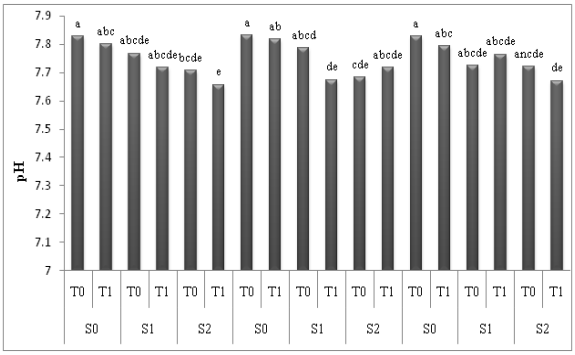

Fig. 1. Comparison of the average acidity of the compound manure application (K0, K1 and K2, respectively, without manure, 10 and 20 tons per hectare), sulfur (S0, S1 and S2, respectively, without sulfur, 250 and 500 kg per hectare) and bacteria Thiobacillus (T0 and T1 without the bacteria, germs) A similar character in each column is no difference significant (Duncan test at the 5% level).

Soil Available Potassium

The results showed the use of manure and K has a significant effect on the amount of sulfur (0.01> P) and the other treatment combination was no significant difference (Table 3). The highest rate of soil available K was observed with the use of 20 tons per hectare of manure (with an average of 583.22 mg) followed by treatment of 10 tons per hectare and without manure (mean 466.27 and 395.33 mm/ kg, respectively (table 4). factor of 500 kg per hectare of sulfur with an average of 549.44 mg represented maximum amount of soil available K and showed excellence compared to 250 kg sulfur and no sulfur treatments, with an average of 467.83 and 427.56 mg, respectively (table 4).

In general, application of organic manure increased the concentration of many elements in soil. On the other hand, by decomposition of organic materials, processes such as oxidation of sulfur helped to the soil acidification topically and cause the dissolution and mobility of nutrients in the soil solution as an available form (McBride, 1995).

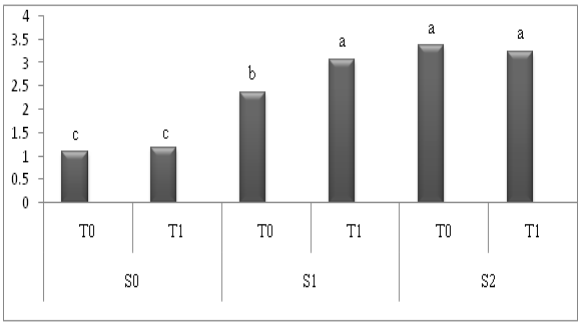

Fig. 2. Comparison between the average zinc concentration in soil treatment combination of sulfur (S0, S1 and S2, respectively, without sulfur, 250 and 500 kg per hectare) and Thiobacillus bacteria (T0 and T1 without bacteria, bacteria).

Manganese concentrations in soil

Results showed significant difference between treatments manure and sulfur based on amount of manganese in the soil (0.01> P) There is no significant change in other sources (Table 3). The consumption of 20 tons per hectare of manure with an average of 12.26 mg/kg was maximum effect on amount of amount of manganese in soil against the treatment of 10 tons and without manure with an average of 10.89 and 10.44, respectively (Table 4). Consumption of 250 kg sulfur per hectare with an average of 75/14 mg/kg represented maximum amount of soil manganese compared to 500 kg and without sulfur with an average of 10.80 and 8.04 mg/kg, respectively (table 4).

Manganese is absorbed into the soil as Mn^{+2} form. In soils with low in organic matter content, high pH and limestone, the manganese availability is low. Manganese dioxide is the most stable oxide of manganese in soil and by soil acidification, its solubility is added. Other forms of manganese such as magnesium carbonate, magnesium silicate hydroxide and oxide are all more soluble and their solubility inversely with soil acidity. As a result of the oxidation of sulfur and sulfuric acid consumption, causing supply needed sulfate reduction in plants and soil pH and increase the ability to absorb phosphorus and micronutrients in the soil (Dawood*et al.*, 1985).

Iron concentrations in soil

Application of Sulfur and *Thiobacillus* bacteria in this study, showed significant effect on the amount of iron at the one and five percent, respectively and other factors as well as their combinations had no significant effect (Table 3). Use of 500 and 250 kg/ha sulfur represented highest amounts of Iron with an average of 6.91 and 6.71 mg/kg, respectively, in comparison to treatment without sulfur with an average of 6.28 mg/ kg (Table 4). *Thiobacillus* bacteria with an average of 6.74 mg compared with no use of bacteria with an average 6.53 mg demonstrated superior absorption rate of iron (Table 4).

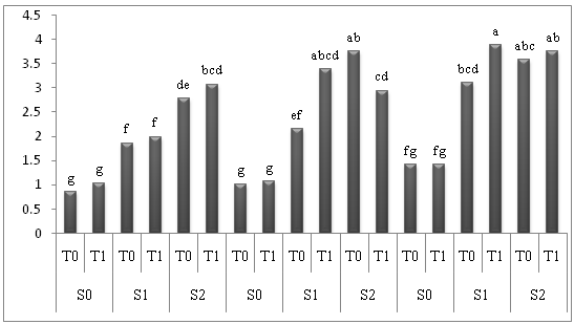

Fig. 3. Comparison of mean serum zinc in soil compounds manure application (K0, K1 and K2, respectively, without manure, 10 and 20 tons per hectare), sulfur (S0, S1 and S2.

Kalbasi*et al.*, (1988) gave similar results regard to sulfur application. They experiment with adding different amounts of sulfur to the soil in the corn, soybean and sorghum and showed that sulfur significantly reduced soil pH and bicarbonate concentration in soil as well as significantly increased available iron, manganese and zinc. Caldwell *et al.*, (1969) reported that by applying 0.5 wt% elemental sulfur in a greenhouse experiment, the amount of available iron significantly improved.

Zinc concentrations in soil

Analysis of variance showed significant differences between application of manure and sulfur regard to amounts of zinc in soil (0.01> P) and the interaction of sulfur and *Thiobacillus* bacteria and triple interactions (manure, sulfur and *Thiobacillus* bacteria) in the 5% was significant (table 3). With increasing use of manure from zero to 20 tons per acre, the amount of soil increased. So that treatments without manure, 10 and 20 t/ha showed an average of 1.92, 2.38 and 2.85 mg respectively, each were placed in separate groups (Table 4). Consumption of 500 kg per hectare of sulfur with an average of 3.31 mg/kg had maximum amount of zinc followed by the treatments of 250 kg of sulfur with an average of 2.72 and sulfur with an average of 1.13 mg/kg so by increasing the amount of sulfur in the soil, zinc was increased (table 4).

Heidarnejad*et al.*, 2012 evaluated effect of sulfur on soils of east Azerbaijan and Khorasan provinces showed that Sulfur has a significant effect on the amount of absorbed zinc so by adding sulfur to the soil, the amount of available Zinc in the soil of East Azerbaijan increased from 0.973 to 0.991mg in the territory of the province and from 0.773 to 0.912 mg. Khan and Schnitzer (1972) report that the concentration of humic organic residues with micronutrients in soil forms complex and increases their availability to plants.

respectively, without sulfur, 250 and 500 kg ha) and Thiobacillus bacteria (T0 and T1 without bacteria, with bacteria).

Copper concentrations in soil

The results of the analysis showed that different factors had no significant effects on soil Cu concentration (Table 3). Although the maximum amount of copper found in the combination of all three factors, but the difference was not significant, which is likely to be due to soil pH is not low enough to release copper or it is maybe because of absence of mineral cupper in surface soil.

Conclusion

To sum up, the results showed that the use of manure was very profitable to supply nutrients needed to the plant macro-elements and also by producing humic acid during the decomposition can formed complexes with micro-nutrients and increases their availability to plants. Animal manure can provide a suitable environment for soil microorganisms that can contribute to soil fertility. The results also showed

that application of sulfur cause increasing availability of some elements such as Phosphorous, Potassium. Manganese, Zinc and Iron.

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