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Maize response to sulfur and thiobacillus inoculation in calcareous soils

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ABSTRACT

Calcareous soils are widely distributed in the arid and semi-arid regions, where most agricultural soils in Iran, due to climatic conditions and geological formations, are calcareous and have a high pH. In such soils, some nutrients like phosphorus, are fixed and utilizing acid-forming substances may increase the availability of this element. Sulfur is considered to be the most affordable acid-producing material and is a byproduct of gas and oil refineries with an annual production of more than two million tons in Iran. In this research, the effects of bentonite-sulfur produced by a new process were tested on maize silage (Single Cross 704 cultivar) on agricultural soil research farms at three sites (Isfahan, Shiraz, and Jiroft) using a factorial experiment. For this purpose, 0, 0.5, 1, and 2 t ha^{-1} of the elemental sulfur as well as 0, 65, and 100% of recommended phosphorus were applied. Application of elemental sulfur was combined with the inoculation of Thiobacillus bacteria (1 kg per 50 kg of elemental sulfur). Results indicated that sulfur, phosphorus, and their co-application brought about significant increases in maize shoot dry and fresh weights only at Shiraz site. Sulfur application enhanced the shoot uptakes of zinc and iron at Shiraz and Jiroft sites. The highest Fe uptake was observed with application of 2,000 kg ha^{-1} of sulfur. No significant effects were, however, detected on shoot phosphorus uptake at any of the study sites. Elemental sulfur was observed to have a limited effect on soil nutrient availability and plant growth because of the high buffering capacity of the studied sites calcareous soils, counteracting the acidification of sulfur oxidation.

Keywords: Maize, Phosphorus , Sulfur, *Thiobacillus*.

1. Introduction

Iran is located in a (semi-)arid region of the world with the average annual rainfall of nearly 250 mm about one-third of the world average while its potential evapotranspiration is three times of the global average (Roozitalab et al., 2018). Calcareous soils constitute considerable portions of the Iranian agricultural lands resulting from dry climate, the parent materials and other factors leading to form this type of soils (Banaee et al., 2004). Regularly in these soils, plant nutrients such as phosphorus, iron, zinc, copper, and manganese are chemicaaly fixed and, therefore, unavailable to plants. The result is, the predominant phosphorus and micro-nutrient deficiencies as the limiting factors in agricultural crop production (Besharati, 2016; Wright et al., 2012). Many studies in the region, have shown that the efficiency of phosphorus fertilizers in calcareous soils does not exceed 20% (Spinks and Barber, 1947; Wiedenfeld, 2011). Acidic amendments have been proposed as a possible approach to overcome this nutrient deficiency and improving plant nutrient uptake in calcareous soils (Schueneman, 2001; Besharati, 2016). Elemental sulfur, as an abundant and most economical acid generating agent, is one of the main strategies within this approach and was employed to increase nutrient availability in calcareous soils (Besharati, 2016). Application of sulfur to these soils lead to oxidation via chemical and biological processes. As a result of sulfur oxidation, H^+ is produced then local soil pHdecrease, especially in the rhizospehere area, so makes nutrients more available to the growing plants (Lindeman et al., 1991; Abdou, 2006; El-Tarabily et al., 2006; Kalbasi et al., 1988; Khadem et al., 2014; Kaplan & Orman,1998). Sulfur application efficiency, however, depends on many factors, including sulfur application rate and soil buffer capacities (Jaggi et al., 2005; Besharati, 2016). High application rates of elemental sulfur to match soil buffering capacities in calcareous soils, however, are not only environmentally unsafe but are also uneconomical. There is, therefore, a strong need to determine the effectiveness of elemental sulfur in conditions with high pH and calcium carbonate levels. Determination of sulfur efficiency requires knowledge of sulfur oxidation rates and the amounts of elements released around plant roots. It is the objective of the present study to determine the effects of elemental sulfur and *Thiobacillus* inoculation on the uptake of some nutrients and maize yield under field conditions. The study takes advantage of the abundance of sulfur in Iran as an available element for agricultural applications as it is a byproduct of the oil and gas refineries producing around two million tons of sulfur every year.

Table 1. The soil analysis results of different regions

Region	ECe*	pHe*	T.N.V	O.C	P	K	Fe	Zn
	dS m^{-1}	–	%			mg kg^{-1}		
Shiraz	1.31	8.10	32.00	0.60	10.50	242	5.0	0.66
Isfahan	4.70	7.30	36.00	0.45	8.00	280	6.0	0.70
Jiroft	3.10	7.60	10.25	0.01	6.25	390	5.7	0.9

*measured at soil saturated extracte

2. Materials and Methods

Field experiments were conducted as a 4×3 factorial randomized complete block design with 3 replications at the research farms of agricultural and natural resources stations in Isfahan (32° 39′ N, and 51° 40′ E), Shiraz (29° 37′ N and 52° 32′ E), and Jiroft (28° 40' N and 57° 44' E) on maize silage cultivar of Single Cross 704. The treatments were soil mixed with elemental sulfur, and included with *Thiobacillus*, and and soil fertilized with triple super-phosphate (TSP). Four levels of sulfur and three levels of TSP were applied as followed:

S0: Control (without sulfur and Thiobacillus)

S1: Application of 500 kg S.ha^{-1} + 10 kg *Thiobacillus* inoculant

S2: Application of 1000 kg S.ha^{-1} + 20 kg *Thiobacillus* inoculant

S3: Application of 2000 kg S.ha^{-1} + 40 kg *Thiobacillus* inoculants

And triple super-phosphate levels were:

P0: Control (without phosphorus application)

P1: Application of Triple super- phosphate fertilizer based on soil test

P2: Application of Triple super-phosphate fertilizer at the rate of 65% of recommendation.

Three replications for each treatment were used. The soil of all three experimental farms had phosphorus concentrations below the critical level for maize cultivation. Fertilizer applications were determined based on soil analysis (Table 1) and maize yield potential in each region. Sulfur and *Thiobacillus* were well mixed together, applied to the plots, and uniformly mixed with soil before furrows were created. Each plot was 36 m^2 including six furrows 10 m in length. The first and sixth furrows were considered as guard lines. Farming operations were accomplished according to the guidelines for each region. Plant water requirements were determined based on the local conditions in each region. Samples were collected at the V7-V8 stage by eliminating one meter from the beginning and the end of each plot. After harvesting, the shoot's fresh and dry weights as well as phosphorus, zinc, and iron concentrations were determined using the recommended methods (Jones and Case, 1990; Kuo, 1996). At all the experimental sites, soil properties (including phosphorus, zinc, and iron concentrations as well as TNV, pH, EC, and OC) were measured (Olsen and Sommers, 1982; Lindsay and Norvell, 1978) (Table 1). Thiobacillus was obtained from the collection of beneficial microorganisms at the Soil and Water Research Institute, Tehran, Iran. Postgate medium was used to prepare *Thiobacillus* inoculants (Postgate, 1966). The final cell density of the inoculants was recorded to be about 107 cfu.g-1 of perlite as the carrier. Statistical analysis were performed using Minitab and SAS programs (SAS Institute, Cary, NC, USA), respectively.

3. Results and Discussion

The soil analysis results for the study areas are reported in Table 1.

3.1. Shiraz site

Mean comparisons of the effects of sulfur and phosphorus applications on yield and nutrient uptake at the Shiraz site are presented in Table 2. Application of sulfur and phosphorus had no significant effects on maize yield, while the integrated use of these two elements significantly increased both yield and shoot fresh weight and Zn concentration. The highest yield (Table 2) was obtained with 500 kg of sulfur and 100 kg of triple super-phosphate per hectare, (only significantly different with the S3P1 treatment).

3.2. Jiroft site

Mean comparisons of the effects of sulfur and phosphorus applications on yield and nutrient uptake at Jiroft site are presented in Table 3. Application of phosphorous fertilizer had a significant effect on phosphorus concentration in maize shoot. The highest yield was obtained with the S3P0 treatment. Application of sulfur and phosphorus had no significant effects neither on fresh and dry yields nor on shoot weight and iron and zinc concentrations (Table 3).

3.3. Isfahan site

Mean comparisons of the effects of sulfur and

Table 2. Mean Comparison of treatments on yield and nutrient uptake in Shiraz

Sulfur	Phosphorous	Fresh Weight	Dry Weight	Iron	Zinc	Phosphorous	Final yield*
		kg m-1	kg m-1	mg kg-1	mg kg-1	mg kg-1	kg 5m-1
	P0	1.52e	0.39a	428.33a	28.66abc	0.21a	28.27ab
S0	P1	1.94a	0.43a	251.66a	29.00ac	0.22a	30.70a
	P2	1.42de	0.69a	260.66a	34.66a	0.21a	28.73ab
	P0	1.62abcde	0.34a	272.00a	33.66a	0.22a	28.80ab
S1	P1	1.29e	0.36a	235.00a	22.00cd	0.21a	26.53bc
	P2	1.81ab	0.45a	227.66a	20.33d	0.21a	28.93ab
	P0	1.61abc	0.36a	243.00a	32.33ab	0.23a	29.46ab
S2	P1	1.45ce	0.36a	237.66a	29.66abc	0.22a	31.53a
	P2	1.78abc	0.42a	254.00a	24.33bc	0.22a	28.50ab
	P0	1.67abcd	0.43a	264.33a	33.66a	0.22a	28.47ab
S3	P1	1.71abcd	0.39a	241.00a	33.66a	0.23a	24.33c
	P2	1.77abc	0.51a	244.00a	31.33ab	0.23a	28.57ab

S0: Control (without sulfur and thiobacillus application), S1: Application of 500 kg $S.ha^{-1}$ + 10 kg Thiobacillus inoculants, S2: Application of 1000 kg $S.ha^{-1}$ + 20 kg Thiobacillus inoculants, S3: Application of 2000 kg S / ha + 40 Kg Thiobacillus inoculants, P0: Control (without P application), P1: Application of Triple super- phosphate fertilizer based on soil test, P2: Application of Triple super-phosphate fertilizer by 65% of recommended

*Since 5 meters of corn row length were harvested and the corn yield was measured, the yield was reported as kilograms per 5 meters of row length.

Table 3. Mean Comparison of treatments on yield and nutrients in Jirotf

Sulfur	Phosphorous	Fresh Weight	Dry Weight	Iron	Zinc	Phosphorous	Final yield*
		kg m-1	kg m-1	mg kg-1	mg kg-1	mg kg-1	kg 5m-1
	P0	4.43a	0.79a	168.16 ab	41.62a	0.15a	29.61a
S0	P1	4.50a	0.82a	128.76 bc	29.41a	0.15a	37.21a
	P2	5.63a	0.98a	163.72 ac	39.39a	0.15a	33.01a
	P0	4.70a	0.86a	130.97 bc	38.85a	0.15a	34.00a
S1	P1	6.43a	1.12a	97.12 bc	27.19a	0.14a	39.03a
	P2	4.63a	0.86a	106.5 bc	29.97a	0.14a	29.94a
	P0	5.33a	0.89a	143.15 ac	27.74a	0.12a	33.32a
S2	P1	6.73a	1.07a	218.09 a	30.52a	0.14a	37.73a
	P2	4.73a	0.89a	150.38 ac	36.63a	0.14a	32.58a
	P0	6.33a	0.96a	111.53 bc	29.41a	0.15a	40.28a
S3	P1	6.73a	1.17a	78.25 c	34.96a	0.11a	40.14a
	P2	6.40a	1.03a	124.3 bc	38.29a	0.15a	37.86a

S0: Control (without sulfur and thiobacillus application), S1: Application of 500 kg $S.ha^{-1}$ + 10 kg Thiobacillus inoculants, S2: Application of 1000 kg $S.ha^{-1}$ + 20 kg Thiobacillus inoculants, S3: Application of 2000 kg S / ha + 40 Kg Thiobacillus inoculants, P0: Control (without P application), P1: Application of Triple super- phosphate fertilizer based on soil test, P2: Application of Triple super-phosphate fertilizer by 65% of recommended

*Since 5 meters of corn row length were harvested and the corn yield was measured, the yield was reported as kilograms per 5 meters of row length.

phosphorus applications on yield and nutrient uptake at the Isfahan site are presented in Table 4. Sulfur, phosphorous, and their co-application had no significant effects on the measured indices (Table 4).

Conflicting results have reportedly emerged from the many studies conducted to determine the effects of sulfur application on different plants. In this study, the application of sulfur and *Thiobacillus*, either alone or in combination with phosphorus fertilizer, revealed their significant effects on the measured indices. Bromfield et al. (1981) applied ordinary super-phosphate, rock phosphate, rock-phosphate + sulfur (p/s ratio of about 0.3

Table 4. Mean Comparison of treatments on yield and nutrients in Isfahan

Sulfur	Phosphorous	Fresh Weight kg m^{-1}	Dry Weight kg m^{-1}	Iron mg kg^{-1}	Zinc mg kg^{-1}	Phosphorous mg kg^{-1}	Final yield* kg 5m^{-1}
	P0	0.24a	0.95a	0.19a	145.00a	78.67a	0.24a
S0	P1	0.24a	0.99a	0.20a	149.00a	69.67a	0.24a
	P2	0.23a	1.00a	0.20a	159.00a	66.00a	0.23a
	P0	0.24a	1.00a	0.20a	128.33a	74.67a	0.24a
S1	P1	0.24a	1.03a	0.20a	124.33a	66.00a	0.24a
	P2	0.24a	1.07a	0.22a	140.00a	71.33a	0.24a
	P0	0.23a	1.08a	0.22a	142.33a	75.00a	0.23a
S2	P1	0.23a	1.05a	0.20a	130.00a	67.33a	0.23a
	P2	0.24a	0.94a	0.19a	140.00a	66.33a	0.24a
	P0	0.24a	1.02a	0.21a	135.00a	70.33a	0.24a
S3	P1	0.23a	1.07a	0.22a	133.33a	69.67a	0.23a
	P2	0.23a	1.02a	0.20a	136.00a	63.00a	0.23a

S0: Control (without sulfur and thiobacillus application), S1: Application of 500 kg S ha^{-1} + 10 kg Thiobacillus inoculants, S2: Application of 1000 kg S ha^{-1} + 20 kg Thiobacillus inoculants, S3: Application of 2000 kg S ha^{-1} + 40 Kg Thiobacillus inoculants, P0: Control (without P application), P1: Application of Triple super- phosphate fertilizer based on soil test, P2: Application of Triple super-phosphate fertilizer by 65% of recommended

*Since 5 meters of corn row length were harvested and the corn yield was measured, the yield was reported as kilograms per 5 meters of row length.

and 1) on a sandy loam soil with low available phosphorus and sulfate. Maize yield in the control was 11.38 t.ha^{-1}, which increased to 18.79, 16.93, 18.10, and 17.17 t.ha^{-1} in the above-mentioned treatments, respectively. They claimed that oxidation of sulfur in the rock phosphate/sulfur mixture produced sulfate and acid not only to supply the sulfate required by the plant but to dissolve rock phosphate and release P for plant uptake as well; hence, the ultimate increase in maize yield.

Besharati (2016) applied elemental sulfur to calcareous soils with 23%, 17%, and 8.5% Total Neutralizing Values (TNV). The highest grain yield, biological yield, plant height, and shoot Fe and Zn concentrations were obtained with sulfur application rates that neutralized 2% and 4% of soil TNV. The results also revealed that sulfur application along with *Thiobacillus* was able to increase nutrient availability, nutrient uptake, and yield in wheat grown in calcareous soils. Singh & Chhibba (1991) reported that application of 20 mg of sulfur per kg increased yield and sulfate uptake in maize and wheat crops compared to their controls(. It was observed in the present study that shoot nutrient uptake was not affected by the treatments at some of the experimental sites. In a similar research, Mahler and Maples (1986) reported that sulfur application decreased phosphorus uptake in wheat crop, but that manganese, iron, and zinc uptakes followed no specific trends. Singh and Chaudhari (1997) found that the yield of groundnut grown in a calcareous soil increased by 8.6–9.8% compared to the control since application of sulfur increased its iron uptake.

Application of 20, 30, 40, and 50 kg of sulfur per hectare reportedly increased soybean dry weight by 17.44, 29.65, 29.9, and 32.32%, respectively, compared to the control (Dubey & Billore, 1995). Scherer and Lange (1996) reported that sulfur applied at 20 mg.kg^{-1} resulted in the highest N_2 fixation and yield in pea, alfalfa, clover, and mung bean plants. Application of a mixture of sulfur, phosphorous, compost, and *Thiobacillus* inoculants to a sorghum planatation revealed that sorghum yield under these treatments did not differ greatly from that under the triple super-phosphate fertilizer (Rosa et al., 1989).

Rock phosphate (RP), bio-fertilizers produced with sulfur and *Thiobacillus* (Biof1, Biof2, and Biof3), rock phosphate with sulfur (10, 15, and 20%) without *Thiobacillus* (Nbiof1, Nbiof2 and Nbiof3), and Triple Super-Phosphate (TSP) were applied to a calcareous soil with low available P. Higher shoot dry matter, total P, and shoot Fe and Zn concentrations were observed when biofertilizers with sulfur and *Thiobacillus* (Biof) or Triple Super-Phosphate (TSP) were applied. Moreover, compared with the control, these two treatments were found to increase significantly the values for other plant parameters (Besharati et al., 2007).

Phosphorus fertilizer and sulfur *Thiobacillus* sp. increased canola oil production by a maximum of 548 and 335 kg.ha^{-1}, respectively. P-solubilizing bacteria (*Bacillus* sp.) and *Thiobacillus* sp. enhanced the uptake of nitrogen, phosphorous, potassium, zinc, and manganese (Salimpour et al., 2012). Although soil TNV is a major factor limiting the availability of pH-sensitive nutrients, it is not the only determining parameter; rather, other soil characteristics that play roles in sulfur oxidation, counteracting acidification of S oxidation, and releasing fixed nutrients from the soil are also important in sulfur application efficiency.

4. Conclusion

Almost all the maize cultivated lands in Iran are calcareous; hence, the phosphorus and micro-nutrient deficiencies commonly observed in maize. On the other hand, application of micro-nutrients and phosphorus-containing fertilizers on such soils has mostly led to very low efficiencies. An economical solution is to apply sulfur, as an acid-producing agent, in calcareous soils to meet nutrient deficiencies in crop plants. This has, however, often yielded controversial results. The reasons for the inconsistencies might be related to differences in crop management practices, soil characteristics, and climate conditions. Elemental sulfur was observed to have limited effects on soil nutrient availability and plant growth because of the high buffering capacity of these calcareous soils that counteracted the acidification of sulfur oxidation. This is while application of higher amounts of elemental sulfur was observed to enhance nutrient-availability in soil and, thereby, plant yield.

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