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Effect of *Thiobacillus*, sulfur, and vermicompost on the water-soluble phosphorus of hard rock phosphate

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ABSTRACT

Sulfur, organic matter, and inoculation with sulfur-oxidizing bacteria are considered as amendments to increase the availability of phosphorus from rock phosphate. The present study was conducted to evaluate the best combination of sulfur, vermicompost, and *Thiobacillus thiooxidans* inoculation with rock phosphate from Yazd province for direct application to agricultural lands in Iran. For such study, an experiment was carried out in a completely randomized design with factorial arrangement: Elemental sulfur originated from Sarakhs mine at three rates, 0% (S1), 10% (S2), 20% (S3), vermicompost at two rates, 0% (V1), 15% (V2), and inoculation without (B1) and with (B2) *T. thiooxidans*, in three replications. The results showed that water-soluble phosphorus (WSP) content was significantly higher in inoculated treatments compared to non-inoculated treatments. Sulfur had a significant effect on WSP. The highest solubility rate of rock phosphate was obtained in 20% of sulfur (S3) treatments and it was 2.4 times more than S1 treatments. Vermicompost also had a significant and positive effect on WSP of rock phosphate dissolution. The results also revealed that the highest concentration of WSP, sulfate and the lowest pH were obtained in treatments with 20% sulfur, 15% vermicompost inoculated with *T. thiooxidans* (B2S3V2).

1. Introduction

Phosphorus (P) is one of the most important essential elements for crop production. Most of the soils in Iran are P deficient or marginally deficient (Besharati et al., 2001; Vassilev et al., 2001). Phosphorus element has routinely been applied to agricultural lands in Iran since 1946 (10 tons for the first year in Karaj). The current annual consumption of phosphate fertilizers in Iran is approximately 750 thousand tons, about 250 thousands of which are produced in the country and the rest are imported (Besharati et al., 2001). Phosphorus fertilizers, such as single superphosphate (SSP) or triple superphosphate (TSP) are used for different crops such as wheat, barley, maize and they have the potential to supply phosphorus to plant immediately after application. The high cost of single or triple super phosphate fertilizer production has generated considerable interest toward direct utilization of rock phosphate in some countries as well as Iran (Besharati et al., 2001). However, direct application of rock phosphate is not a common practice in the country because of its low solubility in calcareous soils. Solubility of phosphorus in hard rock phosphates may increase by grinding or applying it in acidic soils (Rajan and Watkinson, 1992). They also can be mixed with chemicals that produce acids (e.g. elemental sulfur, pyrites, $(NH_4)_2SO_4$, NH_4NO_3) in non-acidic soils. Sulfur oxidation also increases the availability of phosphorus from hard rock phosphates in calcareous and alkaline soils by reducing the soil reaction (Stamford et al., 2003). In this process, sulfur is biologically oxidized and converted to sulfuric acid. The availability of phosphorus and other elements are affected by sulfuric acid.

Numerous studies have been conducted to evaluate different soil amendments in order to increase the availability of phosphorus from rock phosphates (Ghani et al., 1994; Stamford et al., 2003). Organic matter (Nishanth and Biswas, 2008; Odongo et al., 2007) and sulfur applications (García de la Fuente et al., 2007; Rajan, 1983) and bacterial inoculation (Chi et al., 2007; Muchovej et al., 1989; Sahu and Jana, 2000; Stamford et al., 2003) are considered as important amendments in rock phosphate application.

In the present study the effect of *Thiobacillus*, sulfur, and vermicompost on water-soluble phosphorus (WSP) of hard rock phosphate was investigated in order to find the best combination of examined factors.

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2. Methods

The experiment was arranged in a completely randomized factorial design, with four factors included: powdered elemental sulfur (particle diameter <0.6 mm) obtained from Sarakhs mine located in Khorasan Razavi province at three rates 0% (S1), 10% (S2), and 20% (S3) (Stamford et al., 2003), vermicompost (prepared from cow manner) at two rates 0% (V1), 15% (V2), non-inoculation (B1) and inoculation with *Thiobacillus thiooxidans* (B2) and incubation time (2, 5, 15, 25, 35, and 60 days). All treatments were replicated three times with a total of 144 samples. 1 kg of air-dried rock phosphate was placed in plastic bags (20 cm width, 25 cm length). With a total 144 kg of rock phosphate applied. Mature vermicompost (prepared from cow manure, 1 mm size) was used in the experiment.

Bacterial cultures were ordered from the Research Institute of Soil and Water in Tehran. Mixtures were incubated under aerobic conditions for 60 days. During the incubation time, boiled, filtered water was added to the samples to keep them at 50% of water holding capacity, monitored by daily weighing.

All treatments were thoroughly mixed with the rock phosphate and prepared uniformly. Half of the samples were inoculated by *T. thiooxidans* suspension (80 ml of 10^6 cell ml^{-1} culture for each sample). Treatments were incubated at 25 °C for 60 days. Table 1 shows the chemical analysis of cow vermicompost and rock phosphate. Organic carbon of vermicompost was determined using the method described by Walkey and Black (1934). Nitrogen (N) content was determined by micro-kjeldahl method according to Bremer (1960), Water-soluble phosphates (WSP) of treatments were measured by the chlorostannous reduced molybdophosphoric acid blue method described by Jackson (1967). pH of rock phosphate and electrical conductivity (EC) was measured by a pH meter model METROHM Model 632 and JENWAY 4310, respectively, at 0, 5, 15, 25, 35, and 60 days after incubation. Sulfate content was measured at 0, 15, 35, and 60 days after incubation using spectrophotometer model WPA-S200 at 420 nm wavelength. The MSTATC software was used for statistical analysis of data and means were compared by the least significant differences (LSD) test ($p \leqslant 0.05$). All statistical analyses and correlations were performed using the SAS Software.

3. Results and discussion

Table 2 shows the analysis of variance of the experiment. The results demonstrated that sulfur, vermicompost, *T. thiooxidans*, and time had a significant effect on WSP, pH, and SO_4^{-2} contents of all treatments.

Inoculation of rock phosphate with *T. thiooxidans* showed a significant effect at $p < 0.05$ on WSP (Fig. 1). From the second day of incubation, WSP increased remarkably in all inoculated (B2) treatments. The increasing rates of WSP in B2 treatments were higher than non-inoculated treatments (B1). The highest concentration of WSP was obtained at 25th and 60th day of incubation for B2 and B1 treatments respectively. After 60 days of incubation, the concentration of WSP in B2 was 3.8× higher than B1 treatments.

Table 2
Analysis of variance of treatments.

Source	Degree of freedom	Mean square		
		WSP	pH	SO_4^{-2}
S	2	2355374.44*	107.19*	186671.7*
V	1	2101933.41*	20.15*	94560.36*
SV	2	61315.4*	88.91*	26792.99*
B	1	21105358.17*	83.96*	119916.8*
SB	2	446281.9*	300.92*	14444.06*
VB	1	89487.54*	208.38*	14083.76*
SVB	2	316101.58*	85.97*	6221.38*
T	5	2399035.9*	42.81*	121775.8*
ST	10	167770.48*	226.46*	27790.74*
VT	5	122727.34*	50.36*	12842.65*
SVT	10	12801.556*	16.87*	7762.81*
BT	5	1105398.06*	142.33*	12727.78*
SBT	10	37400.25*	30.04*	2281.68*
VBT	5	32673.88*	10.38*	1657.17*
SVBT	10	27367.65*	6.84*	1210.66*
Error	144	296.72*	4.79*	17.84*
CV%	2	2355374.44*	107.19*	186671.7*

* Significant differences at $p < 0.05$.

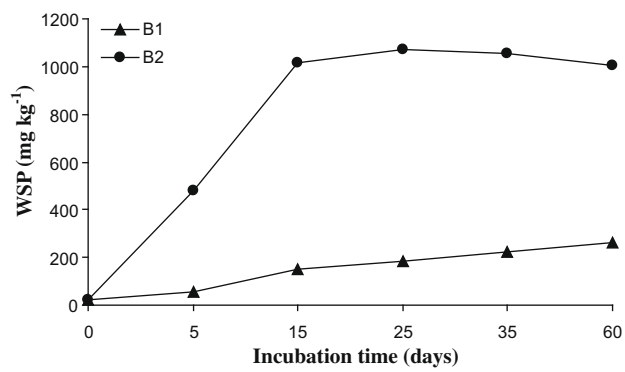

Fig. 1. Effect of incubation of *Thiobacillus thiooxidans* (non-inoculated B1 and inoculated B2 treatments) on WSP of rock phosphate during 60 days of incubation.

The results also revealed that 15–25 days of incubation was enough for reaching a maximum concentration of WSP in B2 treatments under laboratory conditions. Shata et al. (1992) found that application of sulfur combined with a mixture of autotrophic sulfur oxidizing bacteria increased wheat and maize tissue phosphorus concentration by 3-fold. Abd-Elfattah et al. (1992) reported that phosphorus uptake by soybean increased by 19.3% as a result of application of sulfur only, whereas application of sulfur in combination with *T. thiooxidans* increased extractable phosphorus to nine times that of untreated rock phosphate.

Fig. 2 shows the results of sulfur application on WSP of rock phosphate. When rock phosphate amended with sulfur, WSP increased significantly. The highest WSP was obtained in S3 treatment (20%) and the lowest amount of WSP was observed at S1 treatment (0%). The most significant effect of sulfur on WSP was

Table 1
Chemical properties of vermicompost and rock phosphate.

Substance	EC ($dS\ m^{-1}$) (1:1)	pH (1:1)	Sulfate ($mg\ kg^{-1}$)	Total P_2O_5 (%)	Soluble P ($mg\ kg^{-1}$)	Total N (%)	Organic C (%)
Vermicompost	1.45	7.5	ND	1.15	43.75	1.2	10.92
Rock phosphate	0.7	7.6	368.5	37.5	19.5	ND	ND

ND: not detected.

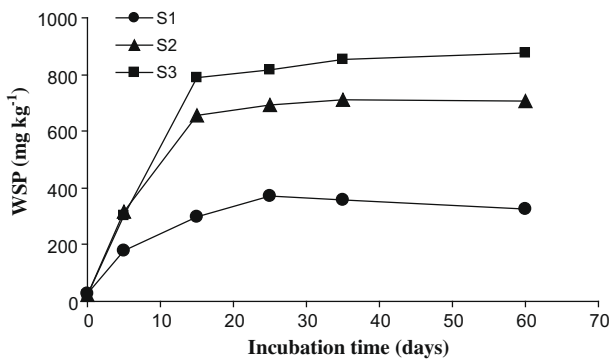

Fig. 2. Effect of sulfur application (S1 0%, S2 10%, and S3 20%) on WSP of rock phosphate during 60 days of incubation.

observed until the 15th day of incubation. Since then, no remarkable changes ware observed. The concentration of WSP in S1, S2, and S3 treatments were 358, 711, and 835 mg kg^{-1}, respectively. The results also revealed that WSP of rock phosphate increased in S1 treatments during the incubation time and the WSP concentration reached to 358 mg kg^{-1} at the end of experiment. Evans et al. (2006) reported that co-treatment of rock phosphate with sulfur was necessary to increase Olsen phosphorus which was generally associated with increased plant dry matter. They also reported that the amount of required sulfur was less in acidic soils. Ghani et al. (1994) reported that treatments without elemental sulfur or *Thiobacillus* had little effect on the dissolution of rock phosphate. The combination of elemental sulfur and *Thiobacillus* increased extractable phosphorus to nine times greater than that of untreated rock phosphate.

Vermicompost also showed a significant effect on the WSP of hard phosphate rock. The most important changes in the concentration of WSP occurred during the first 35 days of incubation and then the variation in WSP was almost constant until the end of incubation period (Fig. 3). The concentrations of WSP in treatments without (V1) and with (V2) vermicompost were 476 and 796 mg kg^{-1} after 60 days of incubation, respectively. Odongo et al. (2007) reported that phosphorus from rock phosphate could be mobilized by using composting manure. They also reported that wheat straw attributed to phosphorus release from rock phosphate as a result of mineralized organic compounds produced during composting chelated with Ca from rock phosphate to increase available P. This is in agreement with Kpomblekou and Tabatabai (2003) who found that phosphorus release was negatively correlated with the equilibrium pH and positively correlated with Ca released from the rock phosphate. Evolved CO_2 during the process of organic manures decomposition results in formation of carbonic

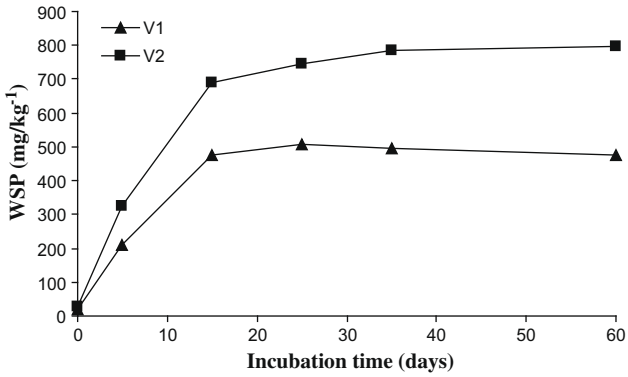

Fig. 3. Effect of vermicompost (V1 0%, V2 15%) on WSP of rock phosphate during 60 days of incubation.

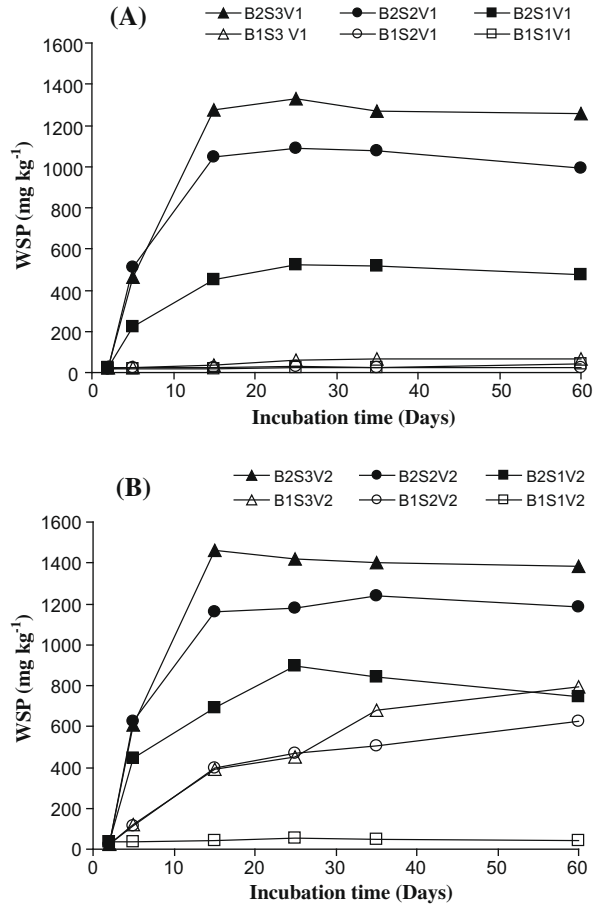

Fig. 4. Interactive effects of *Thiobacillus thiooxidans* and sulfur without vermicompost (A) and with vermicompost (B) on WSP during 60 days of incubation.

acid, which dissolves rock phosphate (Chien, 1979). Their results were in close conformity of the work done by others (Bangar et al., 1989; Biswas and Narayanasamy, 2006; Mathur et al., 1980).

Fig. 4A and B shows WSP changes among all treatments during the incubation time. The increasing trend of WSP in all treatments without vermicompost (Fig. 4A) was similar to those treatments with vermicompost (Fig. 4B). However concentrations of WSP in V1 treatments were lower compared to those of V2 treatments. The WSP of non-inoculated treatment unamended with sulfur (B1S1V2) did not change during incubation time. It was the only treatment (among V2 treatments) that showed a constant WSP

Table 3
Interaction effects of *Thiobacillus thiooxidans*, sulfur, and vermicompost on pH during 60 days of incubation.

Treatment	Incubation time					
	2 days	5 days	15 days	25 days	35 days	60 days
B2S3V2	7.22^{b}	6.14^{e}	3.43^{g}	2.60^{h}	2.26^{h}	2.23^{f}
B2S2V2	7.40^{ab}	6.30^{e}	4.00^{f}	2.97^{g}	2.44^{h}	2.48^{f}
B2S1V2	7.40^{ab}	7.11^{bc}	6.92^{bc}	6.79^{c}	6.72^{c}	6.66^{bc}
B1S3V2	7.28^{b}	6.90^{cd}	5.78^{d}	4.70^{e}	4.36^{e}	3.23^{e}
B1S2V2	7.44^{ab}	6.72^{d}	5.31^{e}	4.51^{e}	3.94^{f}	3.21^{e}
B1S1V2	7.49^{ab}	7.37^{ab}	7.28^{ab}	7.20^{b}	7.16^{b}	7.15^{a}
B2S3V1	7.45^{ab}	5.80^{f}	3.58^{g}	3.20^{g}	3.12^{g}	2.99^{e}
B2SV1	7.50^{ab}	5.87^{f}	3.70^{fg}	3.37^{f}	3.25^{g}	3.23^{e}
B2S1V1	7.53^{ab}	7.24^{b}	7.13^{b}	6.97^{bc}	6.91^{bc}	6.80^{b}
B1S3V1	7.43^{ab}	7.30^{ab}	6.75^{c}	6.23^{d}	5.46^{d}	4.06^{d}
B1S2V1	7.49^{ab}	7.28^{b}	7.03^{bc}	6.98^{bc}	6.91^{bc}	6.39^{c}
B1S1V1	7.67^{a}	7.58^{a}	7.54^{a}	7.49^{a}	7.46^{a}	7.43^{a}

Values with a common letter are not significantly different ($p = 0.05$).

Table 4
Interactive effects of *Thiobacillus thiooxidans*, sulfur, and vermicompost on sulfate concentrations (ppm) during 60 days of incubation.

Treatment	Incubation time			
	2 days	15 days	35 days	60 days
B2S3V2	5177.6[a]	36043.0[b]	91504.0[a]	91104.0[a]
B2S2V2	4023.4[b]	51986.0[a]	58750.4[b]	69658.4[b]
B2S1V2	2245.1[c]	4166.0[gh]	5604.6[i]	6933.1[i]
B1S3V2	4213.1[b]	10628.0[f]	45104.8[c]	61541.6[d]
B1S2V2	3651.0[b]	14320.0[e]	23546.2[f]	33058.4[e]
B1S1V2	1073.0[d]	1540.0[ih]	2478.7[j]	2855.0[j]
B2S3V1	1899.8[c]	27927.0[c]	37299.5[d]	38247.8[c]
B2S2V1	1893.4[c]	23538.0[d]	33604.6[e]	33896.3[e]
B2S1V1	303.8[e]	5141.0[g]	7720.0[h]	11152.2[h]
B1S3V1	1625.9[cd]	8808.0[f]	19799.5[g]	28940.8[f]
B1S2V1	1585.2[cd]	8262.0[f]	20225.1[g]	22703.0[g]
B1S1V1	275.8[e]	375.0[i]	368.5[k]	402.0[k]

Values with a common letter are not significantly different ($p = 0.05$).

during 60 days of incubation. Sulfur had no effect on the WSP of non-inoculated treatments not enriched by vermicompost (Fig. 4A). WSP of these treatments was almost constant during the incubation time. Changes in WSP of inoculated treatments (B2) enriched by sulfur with and without vermicompost, were similar in Fig. 4A and B. The concentration of WSP in B2S3V2 treatment was higher compare to other treatments. The effect of inoculation on WSP was higher compare to vermicompost application when sulfur was not applied.

Changes in pH of treatments were exactly in agreement with the results of WSP (Table 3). Treatments with the highest amount of WSP showed the lowest pH value. The pH in B2S3V2 treatment was 2.23. The effect of sulfur application on pH value emerged after 5 days of incubation and reached to the lowest level at the end of incubation time (60 days). The results showed that by increasing the sulfur application, the pH value decreased.

Treatments with the highest WSP also showed the most sulfate content (Table 4). Increased WSP in presence of sulfur and *T. thiooxidans* was in compliance with other reports (El-Fayoumy and El-Gamal, 1998; Lindemann et al., 1991). Stamford et al. (2003) reported that biofertilizers with sulfur and *Acidithiobacillus* decreased pH values, and showed a clear effect of H_2SO_4 produced by the microbiological reaction of *Acidithiobacillus* bacteria. El-Tarabily et al. (2006) found the highest plant growth when sulfur-oxidizing bacteria were combined with sulfur. Using this treatment reduced soil pH and increased soil SO_4^{-2} level and the uptake of N, S, Fe, Mn, and Zn was increased in maize roots and shoots. The P and Cu uptake in maize shoots was not significant compared to the treatment that received the application of sulfur alone.

4. Conclusions

It can be concluded that water-soluble phosphorus (WSP) content was significantly higher in presence of *T. thiooxidans* compared to non-inoculated treatments. Sulfur and vermicompost had a significant effect on WSP. The highest concentration of WSP, sulfate and the lowest pH were obtained in treatments B2S3V2.

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