International Journal of Agriculture and Agricultural Sciences Vol. 1 (1), pp. 009-012, September, 2013. Available online at www.advancedscholarsjournals.org © Advanced Scholars Journals

Full Length Research Paper

Biosuper as a phosphate fertilizer in a calcareous soil with low available phosphorus

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Accepted 30 April, 2013

Laboratory assays were conducted to produce phosphorus (P) biofertilizers from rock phosphate (RP), applying sulphur at different rates of 10, 15 and 20% and inoculated with *Thiobacillus*. A greenhouse experiment was carried out to evaluate the effect of the biofertilizers in a calcareous soil with low available P from the Qazvin plain of "Qazvin State", grown with corn (*Zea mays L.*). The treatments were: rock phosphate (RP), biofertilizers produced in laboratory with sulphur and *Thiobacillus* (Biof1, Biof2 and Biof3), rock phosphate with sulphur (10, 15 and 20%) without *Thiobacillus* (Nbiof1, Nbiof2 and Nbiof3), Triple Super Phosphate (TSP) and a control without phosphorus. In this experiment, shoot dry matter, total P, Fe and Zn in shoots, and also soil available P, Fe and Zn were determined. Higher rates of measured parameters were obtained from biofertilizers with sulphur and *Thiobacillus* (Biof) and in Triple Super Phosphate (TSP). Biofertilizers with sulphur and *Thiobacillus* (Biof) and TSP increased plant parameters significantly compared with control or rock phosphate.

Key words: *Zea mays,* phosphorus uptake, phosphorus fertilization, corn, *Thiobacillus,* rock phosphate.

INTRODUCTION

The basic material for production of phosphorus (P) fertilizers is phosphoric rocks. The most commonly used is apatite, a non-restorable resource (Stamford et al., 2003). The production of P-soluble fertilizers, such as super phosphate requires higher energy consumption, specific strategies, and conduction of researches for the establishment of efficient and economic use of rock phosphates (Goedert and Sousa, 1989; Stamford et al., 2003). Besides, in calcareous and alkali soils most of P-fertilizers used are fixed, so their efficiency is not more than 20 % (Spinks and Barber, 1947; Tisdale et al., 1993). The immediate utilization of phosphoric rock in the rock form is very restricted because of the low solubility (Oliveria et al., 1977).

Many researchers suggested that a possible and economic way to improve nutrient availability and plant growth in calcareous and alkali soils is the use of acidifying materials such as elemental sulphur (Kaplan and Orman, 1998; Kalbasi, et al., 1988; Singh and Chaudhari, 1997). Sulphur (S) is an essential nutrient for plant growth, which is found in different forms. Elemental S is one of them, which is produced in many countries during petroleum refinery as by-product. Among S-containing fertilizers, elemental S due to some favorable properties such as cheapness, ability to oxidation and acid production, ability to act as fungicide that enhances the productivity and quality of crops is becoming increasingly popular in field crops (Scherer, 2001; Aulakh, 2003; Jaggi et al., 2005). Use of S helps to reduce leaching and run-off losses, leaving prolonged residual effects on the S nutrition of the succeeding crop (Boswell and Friesen, 1993). The biochemical oxidation of the S produces H_2SO_4 which decreases soil pH and solubilizes $CaCO_3$ in alkaline calcareous soils to make soil condition more favorable for plant growth, including the availability of plant nutrients (Linderman et al., 1991), especially P (Deluca et al., 1989). Also application of S to alkaline-calcareous soils could assist in correcting iron chlorosis (Saroha and Singh, 1980; Razeto, 1982; Kalbasi et al., 1986).

Application of S to reclamation sodic and alkaline soils, improve plant nutrients availability in calcareous soils and supplying plant required sulphate would only be efficient when it oxidized to sulphate by soil microorganisms and produces sulphuric acid. A wide variety of soil micro flora is involved in sulphur oxidation, in which *Thiobacillus* bac-

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teria are the most important and common S-oxidizing in agricultural soils (Tabatabai, 1986; Wainwright, 1984).

Studies on the isolation and selection of microorganisms with ability to promote S oxidation in soil and in turn higher solubilization of phosphoric rocks have been carried out in many works (Kapoor, et al., 1991; Pathirathna et al., 1989; Schofield et al., 1981; Bardiya et al., 1982; McCready and Krouse, 1982). The beneficial effects of application of apatite along with sulfur and its oxidizing bacteria (*Thiobacillus*) to enhance nutrient availability (P, Fe, Zn,...) and in turn uptake of these nutrients by plants has been showed repeatedly by many researchers (Pathirathna et al., 1989; Schofield et al., 1981; Bardiya et al., 1982; Swaby, 1975).

It is necessary to evaluate and compare the effects of the application of sulphur inoculated with *Thiobacillus* in plant growth and in soil reaction to P soluble fertilizers and rock phosphate, because the sulphuric acid produced in the biological reaction could act in the rock phosphate solubilization and in the soil reaction reducing soil pH, and that could hamper plant growth (Stamford et al., 2002). This study was carried out to evaluate the effect of biofertilizers produced from rock phosphate (RP) and sulphur inoculated with *Thiobacillus* in a calcareous soil with low level of available phosphorus, with comparison to P soluble fertilizer (Triple Super Phosphate) and rock phosphate on corn shoot biomass, total P, Fe and Zn accumulation on shoot.

MATERIALS AND METHODS

For Biofertilizers production using rock phosphate with addition of elemental sulphur at different rates (10, 15, and 20) inoculated with *Thiobacillus* (Biof.) were conducted in laboratory. Bacterial inocula were grown in 250 Erlenmeyer flasks using Postgate medium (Postgate, 1966) for 5 days at 150 rpm in a horizontal shaker at 28-30°C. Inoculation was applied at a rate of 1 $ml.g^{-1}$ of sulphur. Biofer-tilizer with rock phosphate and sulphur without *Thiobacillus* inocula-tion (Nbiof) was also produced. Before application in greenhouse, both prepared fertilizers (Biof. and Nbiof) were moistened with dis-tilled water to field capacity then stored at $30^{o}C$ for 10 days.

A calcareous soil with low available P was used. The soil was collected in the Qazvin plain located in Qazvin State, South of Tehran. Soil samples (0 - 30 cm layer) were sieved (2 mm), mixed and 3.5 kg of soil placed in each plastic pots (35 × 20 cm). Results from soil analyses (3 replications) are pH (H_2O 1:1) 7.8; ECe 0.86 ($dS\ m^{-1}$); exchangeable cations 18 ($mmolc\ kg^{-1}$); P (Olsen) 4.5 mg kg^{-1}; total N 0.5 g kg^{-1}; organic C 5.7 g kg^{-1}; calcium carbonate equivalent 65 g kg^{-1} ; available Mn, Cu, Fe and Zn 3.4, 1.08, 1.9 and 1.7 mg kg^{-1}, respectively; sand, silt and clay contents 302; 436 and 262 g kg^{-1}, respectively (Table 1).

Then biofertilizers produced in trays, were applied in the greenhouse experiment carried out in pots (8 cm^3), grown with corn (*Zea mayze* L.) The pot experiment was arranged in a completely randomized design with four replications. The 9 treatments were: rock phosphate (RP) in the commercial status, biofertilizers produced in laboratory using rock phosphate with elemental sulphur in the rates 10, 15 and 20% inoculated with *Thiobacillus* (Biof1, Biof2 and Biof3); rock phosphate with sulphur without *Thiobacillus* (Nbiof1, Nbiof2 and Nbiof3); triple super phosphate (TSP); and control no P fertilization (P_0). P fertilization was applied following the maximum recommendation for corn according to soil available P, equivalent to 300 kg TSP ha^{-1}. Fertilizers were suspended in 100 ml distilled water and added to pots.

Seeds of corn were surface sterilized with sodium hypochlorite (2.5%) solution for 5 min then washed 8-times with distilled water. Then seeds were incubated at $30^{o}C$ for 48 h. Five germinated seeds were sown in each pot which contain 3.5 kg of soil. After one week the shoots thinned to 3 per pot. During the growing period (90 days), distilled water was added to keep pots at 80% of field capacity, monitored by daily weighing. Plants were harvested after 90 days of planting. Shoot dry weight, and total P, Fe and Zn in shoots were determined following Jones et al. (1991).

At the end of the experiment, after plant harvesting, pH (1:1), available P (Olsen and Sommers, 1982), and available Fe, Zn and Mn (Page, 1982) were determined. All data obtained from shoot dry weight, and total P, Fe and Zn in shoots were analyzed by analysis of variance (ANOVA) and treatments means were separated by the Tukey's test (P = 0.05) with the SAS statistical package (SAS Institute, 1988).

RESULTS AND DISCUSSION

Shoot dry matter of corn were not different under fertilization with either rock phosphate with sulphur and *Thiobacillus* (Biof). Triple super phosphate (TSP) showed higher dry biomass of shoots compared to the others P treatments (Table 2). Biofertilizer with sulphur without *Thiobacillus* (NBiof) yielded greater corn shoot dry matter than the rock phosphate (RP) and control treatment without P fertilizer (P0). But among the biofertilizers with sulphur without *Thiobacillus* (NBiof), only the Nbiof3 had no significant difference with TSP, whereas biofertilizers with sulfur and *Thiobacillus* (Biof) Biof2 and Biof3 had no significant difference with TSP. Phosphorus application affected total phosphorus yields in corn shoot dry matter, and in the absence of phosphorus (P_0), corn grew poorer and accumulated lower amount of total P, although rock phosphate (RP) and biofertilizer with sulphur without *Thiobacillus* (NBiof) produced no significant response to total P accumulation, in comparison to control treatment without application of phosphorus.

Among the biofertilizers, only the Biof3 had significant difference with control from aspect of total P accumulation (Table 3). So total P accumulation in shoots of corn produced significant response when the phosphorus sources were applied, especially with application of biofertilizers with sulphur and *Thiobacillus* (Biof) and triple super phosphate (TSP). Rock phosphate (RP) and biofertilizer without *Thiobacillus* (NBiof) (except for the case of Nbiof3) were not different compared to control treatment without phosphorus application (Table 3). Biofertilizers with sulphur and without *Thiobacillus* (NBiof) led to greater total phosphorus accumulation, probably because of the presence of native sulphur oxidizing microorganisms especially *Thiobacillus* bacteria which, during the long experimental period (90 days) could produce sulphuric acid sufficient to promote phosphorus solubilization of rock phosphate. While in the biofertilizers with sulphur and *Thiobacillus*, the sulphur bacteria *Thiobacillus* elicits the reaction of sulphur with water and oxygen,

Table 1. Some important physico-chemical characteristics of the soil used in this study.

Available (mg kg-1)					OC	T.N.V	Clay	Silt	Sand	CEC	ECe	pH
Zn	Fe	Cu	Mn	P	(%)	(%)	(%)			(Cmolckg-1)	dS m^{-1}	1:1
1.7	1.9	1.08	3.4	4.5	0.57	6.5	26.2	43.6	30.2	18	0.86	7.8

Table 2. Effects of P treatments on shoot dry weight, total P, Fe and Zn in shoot dry biomass of corn grown in a calcareous soil with low available P.

P-sources*	Shoot dry weight (g pot^{-1})	Total P in shoot dry, weight (mg pot^{-1})	Total Fe in shoot dry weight (mg pot^{-1})	Total Zn in shoot dry weight (mg pot^{-1})
Control (P_0)	7.23b	5.32b	0.85b	0.19c
RP	7.36b	5.43b	0.79b	0.18c
Nbiof1	7.43b	5.58b	0.91b	0.21bc
Nbiof2	7.79b	5.73b	1.07ab	0.23bc
Nbiof3	8.22ab	6.11ab	1.34ab	0.31b
Biof1	7.58b	5.94b	1.08ab	0.28b
Biof2	8.05ab	6.47ab	1.37a	0.37ab
Biof3	8.83ab	7.06a	1.53a	0.39ab
TSP	9.81a	7.45a	1.65a	0.43a
CV(%)	5.90	6.65	9.11	7.93

*****Biof = rock phosphate plus sulfur inoculated with *Thiobacillus,* and NBiof = rock phosphate plus sulfur without *Thiobacillus*.
Values followed by different letters are different (P = 0.05), using the Tukey test.

Table 3. Available P (Mehlich 1), Fe, Zn Mn and pH pot trials one day after plant harvest

P-sources*	pH	Available (mg kg^{-1})			
	(1 :1)	P	Fe	Zn	Mn
Control (P_0)	7.8a	4.7c	1.9a	1.7a	3.4a
RP	7.9a	4.6c	1.7a	1.8a	3.3a
Nbiof1	7.8a	4.8c	1.8a	1.6a	3.4a
Nbiof2	7.7a	5.3bc	1.8a	1.6a	3.6a
Nbiof3	7.6a	6.4b	1.8a	1.7a	3.5a
Biof1	7.7a	4.4c	1.6a	1.5a	3.6a
Biof2	7.8a	6.1b	1.7a	1.8a	3.5a
Biof3	7.6a	8.1ab	1.9a	1.8a	3.8a
TSP	7.6a	10.2a	1.8a	1.7a	3.4a
CV(%)	6.2	5.3	9.5	5.8	6.8

*****Biof = rock phosphate plus sulfur inoculated with *Thiobacillus,* and NBiof = rock phosphate plus sulfur without *Thiobacillus*.
Values followed by different letters are different (P = 0.05), using the Tukey test.

forming higher amounts of sulphuric acid (Garcia, 1992) at varying rates, as related to the different amounts of elemental sulphur applied. The sulphuric acid produced reacted with the rock phosphate increasing the available P and lowered pH near plant roots, according to the amount of sulphur in the different biofertilizers and depending on the soil buffering capacity, with consistent results. The effects of the P treatments on shoot biomass compared with applying rock phosphate (RP) in the commercial status and the control treatment without applying P are conclusive. Klepker and Anghinoni (1995) studying the effect of phosphorus application in maize, reported greater response of soluble fertilizers compared with rock phosphates.

Application of P caused a marked increase in total P in plant shoots, and best results were obtained when biofertilizers with rock phosphate with sulfur and *Thiobacillus* and triple super phosphate were used. The positive impact of the biofertilizers produced with rock phosphate plus sulphur inoculated with *Thiobacillus* on total P accumulated in shoots of corn holds great promise for improving input from these products as an alternative for partial or total substitution of soluble fertilizers. Lombardi (1981) observed effect of “Alvorada” rock phosphate applied with sulphur and *Thiobacillus* on P total and growth of a tropical grass. Native bacteria in soil promoted sulphur oxidation as effective as the inoculated bacteria. However, the coefficient of variation obtained in the experiment was so high that it was not possible to evaluate the positive effect of the sulphur inoculation with *Thiobacillus* when compared with the soil bacteria. In this research, the native bacteria present in soil were not effective in the oxidation of sulphur applied in the biofertilizer without *Thiobacillus*. Thus it seems that 90 days after the planting the soil bacteria may produce sulphuric acid and could act in P solubilization increasing available P in soil. Probably the reaction for sulphuric acid production was lowered due to the low input of air and water to react with the sulphur into the rock phosphate. Also Stamford et al. (2002), using sulphur inoculated with *Thiobacillus* in amendment of saline and sodic soils observed reduction in soil pH occurring until the total consumption of the added sulphur, promoting soil acidification varying from initial pH 8.2 up to pH 4.5 applying 1.8 t ha^{-1} of sulphur.

Schofield et al. (1981) assessed biosuper (phosphate rock combined with elemental sulphur and *Thiobacillus thiooxidans*) as a fertilizer on three soils in glasshouse by measuring dry matter yield and P uptake by white clover grown in pots. Biosuper increased DM yields of white clover by 10 - 20% on all three soils. On two soils, the increased yield was similar to that resulting from superphosphate applications at equivalent rates of Whitehouse and Strong (1977) compared biosuper with superphosphate as a phosphatic fertilizer for wheat on two phosphorus deficient soils and concluded that superphosphate increased dry matter production and tissue concentration of phosphorous in both soils while, biosuper had very little or no effect either on yield or phosphorrous uptake. They explained that the biological activity which solubilizes biosuper is a delayed reaction, so more than 4 weeks appears necessary before biosuper is of any value as a phosphatic fertilizer. The solubilization of Mussoori rock phosphate on addition of elemental sulphur and pyrite and on inoculation with *Thiobacilli* bacteria was studied by Kapoor et al. (1991). Sulphur oxidation efficiently solubilized rock phosphate, but the solubilization by pyrite was comparatively less. Inoculation with *Thiobacillus* increased oxidation of sulphur and consequently the solubilization of rock phosphate.

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