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To study the effect of rock phosphate with sulphur levels and biofertilizer on chemical and biological properties of soil

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Abstract

The results indicated that incubation of rock phosphate, along with elemental sulphur, PSB and EM culture showed a significant decrease in soil pH due to elemental sulphur @ 30 and 60 Kg ha⁻¹ along with PSB and EM solution, alone or in combination. A significant decrease in the calcium carbonate content was also observed in these treatments. The results revealed highest available phosphorus content in the rock phosphate treatment + 60 kg S ha⁻¹ + PSB + EM solution. The maximum alkaline phosphatase activity was observed in the rock phosphate treatment with PSB, EM solution and elemental sulphur at 30 days incubation stage.

Keywords: rock phosphate, sulphur, biofertilizer and soil

Introduction

Soybean [*Glycine max.* (L.) Merrill] is one of the most versatile crop, yielding oil and protein across a wide range of the environmental conditions. Soybean belongs to the leguminaceae family. Originated from South East Asia. It is the cheapest and main source of dietary protein of majority vegetarian Indians.

Rock phosphate is one of the basic raw material required for manufacture of phosphatic fertilizers, like single superphosphate, diammonium phosphate and nitro-phosphate etc. Rock phosphate in presence of biofertilizers and soil microflora in the soil is acted upon by certain organic acids which are secreted by the soil microflora. These acids degenerate the rock phosphate to form available mono calcium phosphate.

The use of slow release phosphatic fertilizer, a cheap source of P to plants can be exploited by amending them properly. The dissolution of rock phosphate can be increased by amending them with elemental sulphur. Rock phosphate when added along with elemental sulphur undergoes a series of reactions to finally form monocalcium phosphate. The elemental sulphur undergoes oxidation. The SO₄ ions combine with the hydrogen ions on dissociation of water molecules to form sulphuric acid. The sulphuric acid so formed reacts with the rock phosphate to form monocalcium phosphate, which is an available form of phosphorus. Therefore, direct soil application of indigenous rock phosphate along with P solubilizing culture, EM solution and elemental sulphur can solubilize the insoluble rock phosphate in available form. This will save valuable foreign currency on import of phosphatic fertilizers.

Materials and Methods

The present investigation was carried out with soil incubation and a pot culture experiment to study the phosphorus availability from rock phosphate as influenced by elemental sulphur and biofertilizers.

Soil

The soil selected for present study was of Inceptisol order. The soil at 0-15 cm depth was collected from the Post Graduate Research Farm, dried in diffused sunlight and was pounded and sieved. Three kilogram soil was filled in plastic bowls for the incubation study. The soil moisture was maintained at field capacity during incubation period. The another five kilogram soil from the same locations was filled in earthen pots for the pot culture experiment on

Table 1: Properties of soil used for the experiment

Property	Value
pH (1 : 2.5)	8.2
EC (dSm-1)	0.37
Organic Carbon (%)	0.69
CaCO ₃ (%)	6.25
Available N (kg ha-1)	150.5
Available P (kg ha-1)	15.23
Available K (kg ha-1)	519.68
Soil Sulphur (mg kg-1)	9.047
Total P (mg kg-1)	330.62
AB-DTPA micro-nutrients (µg g-1)	Fe - 4.40, Mn - 4.90, Zn - 0.78, Cu - 0.64
Microbial count (x 10 ⁵ cfu g-1 soil)	Bacteria - 15.30, Fungi - 7.84

2. Rock Phosphate

The fine powder of rock phosphate was analyzed for P fractions (total, citrate soluble and water soluble P₂O₅) and CaCO₃ equivalent by using standard methods of analysis given in Table 2.

Table 2: Characterization of rock phosphate

S. No.	Parameter of Rock Phosphate	P ₂ O ₅ (%)
1	Total P ₂ O ₅	19.17
2	Citrate soluble P ₂ O ₅	1.4
3	Water soluble P ₂ O ₅	-

3. Microbial culture for incubation study

1. Phosphorus solubilizing bacteria – 10 kg PSB/ha through soil
2. EM culture (Effective micro-organism culture) 25 lit drenching/ha through soil at 0,15,30 days
3. EM culture contains Lactic acid bacteria: *Lactobacillus casei*
Photosynthetic Bacteria: *Rhodospseudomonas palustris*
Yeast: *Saccharomyces cerevisiae*

4. Microbial culture for Pot culture experiment

1. Rate of PSB: 250 g/10 kg seed
2. Rate of EM culture: 25 lit/ha through drenching in soil at 0, 15, 30 days

Elemental sulphur three levels

- S0 – 0 kg ha-1 sulphur
S1 – 30 kg ha-1 sulphur
S2 – 60 kg ha-1 sulphur

Incubation study

The incubation study was conducted under ambient climatic conditions in the green house of the Department of Soil Science and Agricultural Chemistry. Plastic bowls of 5 kg capacity were procured and filled with 3 kg, 2 mm sieved soil in twenty eight pots of two replications. The soil in bowls was wetted with water upto field capacity and the moisture was maintained at field capacity. Then rock phosphate was added as per treatment. The soils were mixed thoroughly with rock phosphate, sulphur and bio fertilizer as per treatment. The plastic bowls were kept for incubation for 30 days. The same set of experiment was incubated for 60 and 90 days in two replications each.

The observations were taken by adopting standard procedures. The initial soil sample used in an incubation study was analyzed for pH, electrical conductivity, CaCO₃, organic carbon, available nitrogen, available phosphorus and available potassium, DTPA micro nutrients, total P, microbial count (bacteria and fungi) and soil sulphur. The soil samples from

each treatment were analyzed after 30 and 60 days of incubation for pH, EC, CaCO₃, soil sulphur, alkaline phosphatase enzyme activity, available P and total P content. The soil samples from each treatment were analyzed at 90 days incubation period for pH, EC, CaCO₃, soil sulphur alkaline phosphatase enzyme activity, total P, available P, AB-DTPA, micro nutrients and soil microbial count (bacteria and fungi).

Results and Discussion

Soil properties

pH

A significant decrease in soil pH was observed in the treatments T7 to T14 i.e. rock phosphate along with two levels of sulphur and biofertilizers. It was an overall observation the pH in all the treatment was the lowest in the 30 days incubation stage. A slight non-significant increase in the soil pH was registered in the 60 and 90 days incubation stages over the 30 days incubation stage. This may be due to initial reaction of sulphur and a rapid increase in the microbial population due to the biofertilizer treatments which resulted in lowering the pH at 30 days stage. Slight increase in the pH at 90 days stage may attributed to decreased microbial population (Table 3).

The sulphatase enzyme hydrolysed the esters of sulphur and released SO₄-2 (Havlin *et al.* 2007) [4]. Dubey (2000) also reported a decrease in soil pH due to inoculation of different microbial P solubilizers along with single superphosphate and rock phosphate. The decrease in soil pH might be due to formation of sulphuric acid on microbial oxidation of elemental sulphur, where elemental sulphur was applied.

Electrical conductivity

The effect of incubation of rock phosphate was observed to be significantly affected by different solubilizers (Table 3). The electrical conductivity was observed to increase in the 30 days of incubation over the initial values. The lowest electrical conductivity in all the treatment was observed in the 60 days of incubation. However, the electrical conductivity was again observed to increase at the 90 days of incubation. An initial increase in electrical conductivity might be due to dissolution of rock phosphate and native carbonates of calcium, magnesium and other cations resulting in an increase in electrical conductivity. A decrease in electrical conductivity at 60 days of incubation may be due to partial immobilization by increased microbial population resulting in a slight decrease in the electrical conductivity in all the treatments.

The increase in electrical conductivity in treatment T8 to T14, may be attributed to rapid dissolution of rock phosphate due to elemental sulphur and organic acids secreted by biofertilizers, resulted in an increased electrical conductivity.

Dubey (2000) also reported a slight rise in electrical conductivity in the treatments inoculated with P solubilizing micro organisms.

Calcium carbonate

The calcium carbonate content of soil as affected by different treatments, at 30, 60 and 90 days incubation are presented in Table 3. It was observed that the calcium carbonate slightly increased in the treatments where sulphur was not applied. This might be attributed to the calcium from rock phosphate which reacted with carbonates to form CaCO₃. It was observed that the calcium carbonate content of soil slightly increased in the 90 days of incubation over the 60 days of incubation. Which may be ascribed to the precipitation of solution calcium cations as carbonate of calcium. The calcium carbonate content at 30 days of incubation in all the treatments was observed to be the lowest. This may be due to rapid dissolution of the carbonates of calcium due to

formation of organic acids in the biofertilizer treatments and formation of sulphuric acid by microbial oxidation of sulphur in the elemental sulphur treatments leading to dissolution of the precipitated carbonates of calcium (Yawalkar *et al.*, 1996)^[12]. A slight increase in calcium carbonate content was observed at 60 days of incubation and was the highest in the 90 days of incubation in all treatments. Which may be attributed to reprecipitation of the Ca cations as carbonates. This decrease in the CaCO₃ content of soil might be attributed to microbial oxidation of elemental sulphur, resulting in formation of sulphuric acid, which may have partially dissolved the native calcium carbonate. Kirankumari and Phogat (2008)^[5] reported that the dissolution of CaCO₃ increases the Ca²⁺ concentration in the soil solution, which may be taken up by the plants or leached out. The PSB and EM solution inoculation may also have secreted organic acids, resulting in decrease in the CaCO₃ content of soil.

Table 3: Effect of treatments on pH, electrical conductivity and calcium carbonate of soil during incubation

S. No.	Treatments	pH (1:2.5)			Electrical conductivity (dSm ⁻¹)			Calcium carbonate (%)		
		30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T ₁	Absolute control	8.19	8.20	8.25	0.38	0.31	0.40	6.25	6.3	6.32
T ₂	GRD (50:75:00)	8.21	8.24	8.27	0.40	0.42	0.42	6.62	6.64	6.6
T ₃	Rock Phosphate + S ₀	8.18	8.13	8.19	0.45	0.47	0.51	6.7	6.75	6.77
T ₄	R. P. + S ₀ + PSB	8.16	8.20	8.24	0.45	0.54	0.53	6.68	6.70	6.74
T ₅	R. P. + S ₀ + EM Culture	8.10	8.05	8.09	0.51	0.49	0.54	6.5	6.60	6.71
T ₆	R. P. + S ₀ + PSB + EM Culture	8.10	8.14	8.19	0.49	0.51	0.56	6.37	6.41	6.78
T ₇	R. P. + S ₁	7.89	7.91	8.01	0.67	0.58	0.58	6.0	6.25	6.40
T ₈	R. P. + S ₁ + PSB	7.90	7.96	8.00	0.72	0.69	0.68	5.9	6.0	6.14
T ₉	R. P. + S ₁ + EM Culture	7.88	7.89	7.97	0.78	0.71	0.71	5.8	6.0	6.10
T ₁₀	R. P. + S ₁ + PSB + EM Culture	7.90	7.97	8.01	0.89	0.70	0.74	5.8	6.2	6.28
T ₁₁	R. P. + S ₂	7.75	7.9	7.99	0.93	0.70	0.75	5.6	5.9	6.01
T ₁₂	R. P. + S ₂ + PSB	7.72	7.88	7.87	0.89	0.73	0.76	5.5	6.0	6.10
T ₁₃	R. P. + S ₂ + EM Culture	7.78	7.89	7.90	1.09	0.74	0.75	5.5	5.8	6.10
T ₁₄	R. P. + S ₂ + PSB + EM Culture	7.75	7.84	7.89	1.10	0.75	0.78	5.3	5.4	6.00
	S.E. +	0.093	0.102	0.106	0.0079	0.141	0.0701	0.314	0.120	0.117
	C.D. at 5%	0.280	0.308	0.321	0.241	0.428	0.212	0.951	0.364	0.355

Available sulphur

The soil sulphur content at 30, 60 and 90 days of incubation as affected by different treatments is presented in Table 4. The highest soil sulphur content was observed in the treatment T14 with rock phosphate + 60 kg ha⁻¹ S + PSB + EM culture. The sulphur content was observed to be the highest in the 60 days of incubation. This may be attributed to a gradual oxidation of elemental sulphur in the initial stages, which was maximum at 60 days of incubation and further, slightly decreased in the 90 days of incubation due to decreased microbial activity. The sulphur content was also observed to be higher in the treatment of elemental sulphur at all incubation period and was higher in the 60 kg ha⁻¹ S treatment than 30 kg ha⁻¹ S treatment.

This increase in the soil sulphur content may be attributed to the addition 30 kg and 60 kg ha⁻¹ elemental sulphur in treatments T7 to T10 and T11 to T14 respectively. Trudinger (1967)^[11] reported that the higher availability of sulphur during the initial period of incubation may be due to the induced effect of sulphur oxidizing bacteria. A synergistic S-P effect at low to medium levels of phosphorus with 60 kg ha⁻¹ sulphur was reported by Ali (1991)^[1]. The dissolution of rock phosphate along with elemental sulphur may have resulted in increasing the soil P and soil S levels due to this synergistic interaction.

Available phosphorus

The data presented in Table 4 revealed that the highest

available phosphorus content was recorded in the treatment T2 (general recommended dose) which was significantly superior over rest of the treatment except the treatment T14 rock phosphate + sulphur @ 60 kg ha⁻¹ + PSB + EM solution. The results of incubation study at 90 days as incubation revealed that there was a slight decrease in available phosphorus in all the treatments as compared to the available phosphorus at 30 and 60 days of incubation.

It was observed that the available phosphorus content in the soil at all the incubation period in the general recommended treatment (T2) was the highest (P was supplied through single superphosphate). The available phosphorus content was observed to increase in the treatments of rock phosphate along with PSB and EM solution and elemental sulphur. The available P content was observed to be the highest in treatment T14 (RP + 60 kg ha⁻¹ S + PSB + EM solution) at all the incubation period, which may be attributed to the increased dissolution of rock phosphate due to elemental sulphur and biofertilizers. ROy *et al.* (1999)^[9] observed that the application of phosphobacteria along with Mussorie rock phosphate significantly increased the available P in the soil over control treatment.

Total phosphorus

The data presented in Table 4 revealed that, highest total phosphorus content was recorded in the treatment T2 (general recommended dose) at 30 days of incubation and the results of incubation study at 60 and 90 days showed that, highest

total phosphorus content in soil was recorded in the treatment T14 (rock phosphate + sulphur @ 60 kg ha⁻¹ + PSB + EM solution) and was significantly superior over all the treatments. However, gradual decrease in total phosphorus was observed at 90 days of incubation, in all the treatments over 60 days of incubation.

Alkaline phosphatase activity

The alkaline phosphatase activity (mg P-nitrophenol mL hr⁻¹) was the highest in the T14 treatment i.e. Rock phosphate + S2 (60 kg) + PSB + EM solution followed by treatment T10 Rock phosphate + S2 30 kg + PSB + EM solution at 30, 60 and 90 days of incubation (Table 4). The lowest activity of alkaline phosphatase activity was observed in the 90 days of incubation in all the treatments over the 60 and 30 days of incubation. With increase in the incubation period from 30 to 90 days of incubation a significant decrease in available phosphorus was also observed due to precipitation reaction.

The decrease in the alkaline phosphatase activity at 90 days incubation may be associated with a decrease in microbial population at 90 days of incubation over the 30 and 60 days of incubation.

Olander and Vitousek (2000) observed that phosphorus addition suppressed phosphatase activity. The decrease in the phosphatase activity in the sole treatment of rock phosphate may be due to the above reason. Zahir *et al.* also observed that a significant increase in the alkaline phosphatase activity was observed with increase in mineralization of the phosphate compounds. The results of this investigation are in close conformity with their observations.

Parthasarathi and Ranganathan (2000) [8] reported that the increased activity of the enzyme alkaline phosphatase may be ascribed to the active microbial population and optimal moisture conditions. These observations are in agreement with the observations in this study.

Table 4: Effect of treatments on available sulphur, available phosphorus and total phosphorus of soil during incubation

S. No.	Treatments	Available sulphur (mg/kg)			Available phosphorus (kg ha ⁻¹)			Total phosphorus (mg kg ⁻¹)		
		30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS	30 DAS	60 DAS	90 DAS
T ₁	Absolute control	9.35	10.01	9.40	9.40	10.45	10.40	162.0	159.0	164.0
T ₂	GRD (50:75:00)	20.11	21.64	21.00	18.56	18.90	17.20	324.0	340.2	344.3
T ₃	Rock Phosphate + S ₀	11.35	13.20	13.90	13.18	14.70	13.40	237.0	264.6	267.0
T ₄	R. P. + S ₀ + PSB	12.43	15.70	16.20	13.49	15.10	13.10	242.0	271.8	274.2
T ₅	R. P. + S ₀ + EM Culture	13.50	17.10	17.50	13.45	15.02	14.80	242.0	270.3	268.0
T ₆	R. P. + S ₀ + PSB + EM Culture	14.43	18.70	19.01	13.85	15.30	14.00	249.0	275.4	279.0
T ₇	R. P. + S ₁	21.40	22.70	23.00	13.95	17.78	15.70	254.0	320.04	327.04
T ₈	R. P. + S ₁ + PSB	22.70	27.00	28.40	14.01	15.52	14.90	252.1	333.36	339.3
T ₉	R. P. + S ₁ + EM Culture	23.90	31.00	31.50	13.80	18.61	15.70	253.4	334.9	342.1
T ₁₀	R. P. + S ₁ + PSB + EM Culture	22.90	29.70	30.80	14.10	18.90	16.20	254.3	340.2	347.2
T ₁₁	R. P. + S ₂	31.20	34.60	34.00	14.97	19.20	17.80	269.4	345.6	345.0
T ₁₂	R. P. + S ₂ + PSB	34.50	37.80	38.20	15.71	21.10	17.00	282.8	379.8	381.6
T ₁₃	R. P. + S ₂ + EM Culture	37.40	41.90	42.40	15.65	21.01	19.20	281.7	378.2	384.4
T ₁₄	R. P. + S ₂ + PSB + EM Culture	42.54	42.73	42.73	15.99	22.57	19.70	287.8	400.2	390.0
	S.E. +	1.046	1.324	1.097	0.887	0.949	0.891	1.290	1.785	1.436
	C.D. at 5%	3.166	4.006	3.320	2.685	2.871	2.697	3.905	5.403	4.346

Micronutrient content

DTPA extractable iron

The data presented in Table 5 revealed that there were no significant treatment differences in treatment T1 to T9 and T11 to T13 in the DTPA extractable iron content in the soil at the end of the 90 day incubation stage. The data indicated that the available iron status was observed to be higher in the treatments with rock phosphate along with elemental sulphur PSB and EM solution.

Many micro organisms produce siderophores which are large organic molecules produced by fungi and bacteria and catcholates produced by bacteria which strongly and specifically bind metals especially Fe³⁺ (Fageria *et al.*, 1991) [3].

DTPA extractable Zinc

The data in Table 5 revealed that the highest DTPA Zn was observed in treatment T10, followed by T14, and were at par with each other. The results indicate the bioavailability of Zn in both these treatments in presence of PSB and EM solution. Mandal *et al.* (1988) [6] reported that organic matter and microbial population are responsible for redistribution of Zinc in all fraction of soil indicating an increase in the bioavailability of Zinc.

DTPA Extractable Manganese

On perusal of the data in Table 5, it was observed that there

was an overall decrease in the manganese concentration in all the treatments over the initial concentration. The decrease in the water soluble manganese content at 90 day incubation stage may be due to a higher soil pH at that stage. Sims (1986) [10] reported that the organic and Fe oxide fraction are lower in solubility and are higher at higher pH level. The results of this study are in agreement with the above observations.

DTPA extractable copper

On perusal of the data in Table 5, it was observed that the DTPA extractable Cu decreased at the 90 day incubation stage, over the initial status. Miller *et al.* (1987) [7] reported that copper initially existed in specifically adsorbed forms. After 12 days some Cu was found in the Mn and amorphous Fe oxides, indicating a shift away from plant availability. The results of this study reveal a decrease in DTPA-Cu at 90 day incubation stage, which are in agreement with the observation of Miller *et al.* (1987) [7].

Microbial population

The maximum bacterial and Fungal count was observed in the treatment T14 followed by treatments T10. Amongst all the treatments, it was observed that the treatments with PSB alone and in combination with elemental sulphur (T4, T8 and T12), the bacterial population was slightly higher than the treatments of EM solution alone and in combination (T5, T9

and T13). This may be due to bacterial inoculation of phosphorus solubilizing bacteria (Table 5). Amongst the other treatments, it was observed that the treatment with EM solution alone and in combination with elemental sulphur (T5, T9 and T13), the fungal population was higher than the treatments with PSB inoculation (T4, T8 and

T12) alone and in combination with elemental sulphur. This increase in fungal population in the treatments with EM solution over the treatments with PSB inoculation, may be due to the inoculation of higher fungal population through the effective micro organism solution (EM solution).

Table 5: Effect of treatments on alkaline phosphatase activity, micronutrient status and microbial population of soil during incubation.

S. No.	Treatments	P nitrophenol mg ml ⁻¹ hr ⁻¹			Fe	Mn	Zn	Cu	Bacteria (x 10 ⁵ cfu g ⁻¹)	Fungi (x 10 ⁵ cfu g ⁻¹)
		30 DAS	60 DAS	90 DAS	-----Mg/kg-----					
T ₁	Absolute control	7.80	7.10	6.60	4.38	4.92	0.70	0.60	16.30	9.78
T ₂	GRD (50:75:00)	8.10	7.40	6.70	4.44	4.94	0.82	0.66	24.40	17.54
T ₃	Rock Phosphate + S ₀	7.40	7.80	6.30	4.3	4.50	0.69	0.59	20.40	14.34
T ₄	R. P. + S ₀ + PSB	8.70	8.10	7.90	4.44	4.57	0.62	0.53	42.66	17.64
T ₅	R. P. + S ₀ + EM Culture	8.64	8.20	7.95	4.47	4.61	0.67	0.54	40.40	22.66
T ₆	R. P. + S ₀ + PSB + EM Culture	9.95	9.29	8.70	4.52	4.69	0.79	0.59	69.00	29.86
T ₇	R. P. + S ₁	8.00	7.80	7.40	4.60	4.73	0.64	0.57	30.60	19*.60
T ₈	R. P. + S ₁ + PSB	9.64	9.20	8.85	4.64	4.8	0.63	0.56	39.50	19.84
T ₉	R. P. + S ₁ + EM Culture	9.45	9.20	8.30	4.54	4.72	0.66	0.54	37.20	22.84
T ₁₀	R. P. + S ₁ + PSB + EM Culture	10.20	10.20	9.70	4.85	4.87	0.82	0.59	85.66	32.66
T ₁₁	R. P. + S ₂	8.20	7.80	7.30	4.68	4.64	0.68	0.54	34.00	25.44
T ₁₂	R. P. + S ₂ + PSB	9.70	9.45	9.39	4.69	4.72	0.69	0.57	44.00	32.40
T ₁₃	R. P. + S ₂ + EM Culture	9.54	9.20	8.95	4.67	4.74	0.71	0.57	44.80	34.66
T ₁₄	R. P. + S ₂ + PSB + EM Culture	11.50	11.60	11.00	4.9	4.71	0.78	0.62	93.33	38.66
	S.E. +	0.29	0.17	0.34	0.10	0.23	0.018	0.0136	-	-
	C.D. at 5%	0.88	0.52	1.03	0.33	0.72	0.055	0.0412	-	-

Summary and Conclusions

An appreciable improvement in soil chemical properties was registered in the treatments with rock phosphate sulphur, PSB and EM solution alone or in combination. A decrease in soil pH in these treatments was a significant observation. The available and total P content increased in all the treatments with microbial incubation and elemental sulphur application. The calcium carbonate content in soil can significantly be reduced by application of elemental sulphur and microbial inoculation of PSB and EM solution.

Future line of work

This piece of basic research work is suggestive and can be tried on large scale field trials for validation of the experimental findings, and if proved, rock phosphate, can come up as a cheaper alternative source of phosphorus nutrition for soybean.

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