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Effect of FYM, bio-inoculants and elemental sulphur on inorganic p fractions at critical crop growth stages of maize in calcareous soil

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Abstract
A field experiment was carried out to find out the effect of FYM, bio-inoculants and elemental sulphur on the inorganic P fractions of the soil and the availability of phosphorus at the critical crop growth stages of maize at the glass house of Department of soil science and agricultural chemistry of Tamil Nadu Agricultural University, Coimbatore. The treatments involved two factors namely FYM at four levels (0, 50, 100 and 150 percent of recommended dose) and Phosphate solubilising treatments (PSTs) three types (*Bacillus megaterium, Pseudomonas chlororaphis* and elemental sulphur at 0.5 t ha^{-1} with *Thiobacillus thiooxidans*). The results revealed that the FYM applied at 150 percent has enhanced all the fractions of P at tasseling and maturity stage of the crop and also improved the available P compared to other treatments. It is concluded that the application of higher dose of FYM along with bio-inoculants could enhance the P availability to the crops.

Keywords: P fractions, available P, FYM, *Bacillus megaterium, Pseudomonas chlororaphis*

Introduction
Phosphorous is one among the seventeen essential nutrients and the most important primary plant nutrient which plays crucial role in many physiological processes of plant such as photosynthesis, respiration and is the important component of ATP, the energy currency of the plants. The availability of phosphorus is pH dependent. In alkaline soil, the nutrient is precipitated as ortho calcium phosphates and in acid soils it is adsorbed on the oxides and hydroxides of iron and aluminium.
The total phosphorous in soil constitutes both organic and inorganic P fractions. The total P in Indian soil ranges from 580 to 2900kg ha^{-1} (Tomar, 2000) [27]. The amount of inorganic P fractions in total P ranges from 35 to 70 percent and organic P ranges from 35 to 60 percent (Harrison, 1987) [4]. The inorganic P fractions are saloid P, reductant soluble P, Fe-P, Al-P and Ca-P. Though the soil contain large amount of total P, only very meagre portion of it is available to plants (Keziah *et al.*, 2012) [12]. The P fixation mechanism is due to adsorption, absorption and precipitation of phosphorus with corresponding cations in soil (Zhang and Li, 1998) [34].
Incorporation of FYM increased the available P status of the soil (Kumpawat, 2004) [10]. There was a linear increase in available phosphorus with the application of municipal waste in calcareous soil (Hosseinpur *et al.*, 2012) [5]. Phosphate solubilising microbes could enhance the P uptake by plants through activation of P deposits in soil by solubilisation (Hilda, 2000) [18]. PSB application could increase the PSB population in rhizosphere and thereby enhance the available P status of the soil (Sundar and Natarajan, 2002) [26]. When the energy rich manure is added with rock phosphate and PSB, the phosphate solubilisation tend to become effective (Poi, 1986) [17]. The application of FYM increases the population of PSB and soluble P in the rhizosphere (Sinkha, 1971) [22]. Elemental sulphur at 0.5tha^{-1} resulted in decrease in pH and also has enhanced the availability of P in the soil (Erdal *et al.*, 2000) [2].
The present investigation was carried out to study the effect of FYM, bio-inoculants and elemental sulphur on the inorganic P fractions and availability of P at critical crop growth stages of maize in calcareous soil.

Materials and methods
A pot experiment was conducted in Tamil Nadu Agricultural University, Coimbatore, India in 2018-2019. The soil taken for experiment was alkaline (pH=8.2) and calcareous (free $CaCO_3$ = 13%) in nature. The variety of maize grown was TNAU maize hybrid CO 6. The physicochemical characteristics of the soil are enlisted in the table 1.

The treatments involved two factors namely Farm yard manure (FYM) and Phosphate solubilising treatments (PSTs) which was imposed in FCRD and replicated thrice. FYM was applied at four levels (0%, 50%, 100% and 150%, where 100% FYM = 12.5 t ha^{-1}) and PSTs were three types namely *Bacillus megaterium, Pseudomonas chlororaphis* and elemental sulphur at 0.5 t ha^{-1} with *Thiobacillus thiooxidans.*
The study was conducted in plastic pots with 10 kg of soil with one plant maintained per pot. The pots were maintained separately for each stage for destructive sampling. All the pots received VAM at 2 kg ha^{-1} and N and K_2O @ 118:37.5 kg ha^{-1} (1.8:0.56 g/pot) as urea and muriate of potash, respectively. Phosphorus was applied @ 25% (based on soil test value) of STCR recommended dose i.e., 17kgha^{-1} or 0.26 g/pot P_2O_5 as single super phosphate to all the pots. The bio-inoculants were applied through the seed treatment of maize seeds by treating 1kg of seeds with 6.25ml of each inoculant along with 32 ml of rice gruel respectively. The soil samples were collected at the critical crop growth stages namely tasseling stage (60 DAS) and harvest (110 DAS) for the analysis of inorganic P fractions and available phosphorus. The soil inorganic P fractions were estimated by the method described by Peterson and Corey (1966) [14].

Table 1: Initial soil characteristics of experimental soil

Parameters	Result	Procedure	Reference
Soil texture	Sandy clay loam	International pipette method	Piper (1966) [16]
Soil pH	8.2	1:2.5 soil water suspension	Jackson(1973) [6]
Soil E.C	0.24 dS/m		
Organic carbon	5.5 g/kg	Chromic acid wet digestion	Walkley and Black(1934) [32]
Free $CaCO_3$	14%	Volumetric titration	Piper(1966) [16]
Available N	266 kg/ha	Alkaline permanganate method	Subbiah and Asija(1956) [24]
Available P	20 kg/ha	0.5 M $NaHCO_3$ (pH-8.5)	Olsen *et al.*(1954) [13]
Available K	630kg/ha	Neutral Normal Ammonium Acetate	Stanford & English(1949) [23]
DTPA Zn	0.723 mg/kg		
DTPA Fe	2.72 mg/kg	DTPA extraction and AAS method	Lindsay & Norwell(1978) [11]
DTPA Cu	1.44 mg/kg		
DTPA Mn	16.4 mg/kg		

Results and discussion

The data obtained on the various observations during the course of investigation were analysed statistically by adopting the methods followed for FCRD. The results of the investigation are given below.

Effect of FYM and PSTs on inorganic P fractions

Saloid P

The range of saloid P content at 60 DAS of crop was from 7.66 to 10.5 mg/kg and at harvest it ranged from 7.23 to 9.83 mg/kg (Table 2). Among the treatments of FYM, the highest saloid P was recorded in treatment with 150 percent of FYM (10.15 and 9.33mg/kg at tasseling and harvest stage respectively) and lowest saloid P content was observed in the treatments without FYM (8.0 and 7.44mg/kg at tasseling and harvest stage respectively). The application of 150 percent of FYM registered a significant increase in saloid P in both the stages followed by the application of 100 percent and 50 percent of FYM. This fraction was the least among all the inorganic P fractions evaluated. The slight increase in saloid P might be due to the transformation of applied P and P from FYM into saloid P and Al-P under aerobic conditions in the first instance and then to Ca-P with time (Jain and Sarkar, 1979) [8].
Among the PSTs, *Bacillus megaterium* and *Pseudomonas chlororaphis* performed on par with each other at tasseling (9.28 and 9.30mg/kg respectively) and at harvest (8.58 and 8.49mg/kg respectively) in increasing the saloid P concentration in soil in both the stages. The performance of elemental sulphur was significantly lower (8.57mg/kg at tasseling and 7.99mg/kg at harvest) when compared to that of other two treatments.
The interaction effect of FYM and PSTs were not significant in tasseling stage of the crop but was significant at the harvest stage. In the harvest stage, the interactive effect of *Bacillus megaterium* with FYM at 150 percent significantly recorded higher saloid P (9.83mg/kg) when compared with other treatments. The interactive effect of *Pseudomonas chlororaphis* with FYM at 100 percent and elemental sulphur with FYM at 150 percent were on par with each other in the harvest stage. *Pseudomonas chlororaphis* performed well at optimum dose of FYM (100 percent) in the harvest stage.

Reductant soluble P

The reductant soluble P ranged from 15.2 to 19.3mg/kg at 60 DAS of crop and 14.0 to 18.4 mg/kg at the harvest (Table 3). Among the treatments of FYM, the highest reductant soluble P was associated with the highest dose of FYM (150 percent) in both the stages (18.9 and 18.0mg/kg at tasseling and harvest stage respectively), while the second highest was under 100 percent FYM application followed by 50 percent application. The treatment without FYM recorded the least content of reductant soluble P. It is also in conformity with the findings of Singaram and Kothandaraman, (1992) [20] who observed that if soil is enriched with P through manuring or fertilization, it tend to building up of various P fractions including reductant soluble P.
Among the PSTs, *Bacillus megaterium* and *Pseudomonas chlororaphis* performed on par with each other (17.5 and 17.6mg/kg respectively at tasseling and 16.5 and 16.6mg/kg respectively at harvest) and better when compared to that of elemental sulphur in both the stages. The interactive effect of PSTs with FYM have no significant effect on reductant soluble P in both the stages of the crop.

Fe-P

Fe-P recorded the highest next to Ca-P and the relative concentration of Fe-P was very low as compared to that of Ca-P due to the calcareous nature of the experimental soil. The Fe-P ranged from 18.6 to 24.8mg/kg at tasseling stage and 17.7to 23.8mg/kg at harvest of the crop (Table 4). Among the levels of FYM, application of 150 percent of FYM recorded the highest values of Fe-P (23.4 and 22.4mg/kg at respective stages) in both the stages which was significantly higher at tasseling stage and it was on par with 100 per cent FYM at harvest stage. The increase in Fe-P might be due to the reduction of Fe^{3+} to Fe^{2+}, resulting from the release of organic acids from the decomposition of FYM (Jaggi, 1991) [7] or it might be due to the change in equilibrium of P towards Fe-P (Tripathi and Minhas, 1991) [27].

Among the PSTs, *Bacillus megaterium* and *Pseudomonas chlororaphis* performed on par with each other (21.7 and 21.4 respectively) and better in increasing the Fe-P fractions in soil, when compared to that of elemental sulphur in tasseling stage. In harvest stage, *Bacillus megaterium* performed well (20.8mg/kg) than *Pseudomonas chlororaphis* (20.4mg/kg) and elemental sulphur (19.4mg/kg). The significant effect on the P solubilisation by *Bacillus megaterium* might be due to the production of higher phosphatase enzyme (Turan *et al.*, 2012) [29].

The interactive effect of PSTs with FYM has significant effect on Fe-P fractions at the harvest stage of the crop. The highest values were recorded for the interaction of *Bacillus megaterium* with 150 percent of FYM (23.8mg/kg) followed by *Pseudomonas chlororaphis* with 150 percent of FYM. The lowest values were recorded for the PSTs without FYM.

Al-P

The Al-P content in soil ranged from 14.5to 18.7mg/kg at tasseling stage and 13.5 to 17.9 mg/kg at harvest stage of the crop (Table 5). In both the stages, among the levels of FYM, the highest Al-P was observed in the treatment with the application of 150 percent of FYM (18.6mg/kg at tasseling and 17.6mg/kg at harvest) followed by 100 percent application of FYM and 50 percent. The application of farm yard manure resulted in the production of organic acids thereby, creating localized acidity, resulting in the solubility of Al and formation of Al-P (Viswanath and Doddamani, 1991) [31]. The lowest fraction was observed in the treatment without FYM in both the stages. Treatments with 100 percent FYM and 50 percent FYM are on par with each other in both the stages.

Table 2: Effect of FYM, bio-inoculants and elemental sulphur on Saloid P (mg/kg)

P fraction		**Saloid P**				**Saloid P**		
STAGE		**60 DAS**				**110 DAS**		
FYM	**Phosphate**	**Solubilizing**	**Treatments**		**Phosphate**	**Solubilizing**	**Treatments**	
Levels	***Bacillus megaterium***	***Pseudomonas chlororaphis***	**Elemental sulphur**	**MEAN**	***Bacillus megaterium***	***Pseudomonas chlororaphis***	**Elemental sulphur**	**MEAN**
FYM @ 0%	8.23	8.13	7.66	8.00	7.73	7.36	7.23	7.44
FYM @ 50%	9.06	9.13	8.40	8.86	8.16	8.46	8.00	8.20
FYM @ 100%	9.33	9.76	8.46	9.18	8.60	8.93	7.80	8.44
FYM @ 150%	10.5	10.2	9.76	10.15	9.83	9.23	8.93	9.33
Mean	9.28	9.30	8.57	9.05	8.58	8.49	7.99	8.35
	FYM	PST	FYM×PST		FYM	PST	FYM×PST	
SED	0.124	0.107	0.215		0.108	0.093	0.187	
CD @ 0.05	0.256	0.222	NS		0.222	0.193	0.386	

Table 3: Effect of FYM, bio-inoculants and elemental sulphur on Reductant soluble P (mg/kg)

P fraction		**Rs P**				**Rs P**		
Stage		**60 DAS**				**110 DAS**		
FYM	**Phosphate**	**Solubilizing**	**Treatments**		**Phosphate**	**Solubilizing**	**Treatments**	
Levels	***Bacillus megaterium***	***Pseudomonas chlororaphis***	**Elemental sulphur**	**MEAN**	***Bacillus megaterium***	***Pseudomonas chlororaphis***	**Elemental sulphur**	**MEAN**
FYM @ 0%	16.1	16.0	15.2	15.7	15.1	14.9	14.0	14.6
FYM @ 50%	17.0	17.4	16.8	17.0	16.1	16.3	15.7	16.0
FYM @ 100%	17.7	18.1	16.3	17.3	16.6	17.0	15.5	16.3
FYM @ 150%	19.3	19.0	18.5	18.9	18.4	18.2	17.4	18.0
Mean	17.5	17.6	16.7	17.2	16.5	16.6	15.6	16.2
	FYM	PST	FYM×PST		FYM	PST	FYM×PST	
SED	0.184	0.159	0.319		0.195	0.169	0.339	
CD @ 0.05	0.380	0.329	NS		0.404	0.350	NS	

Table 4: Effect of FYM, bio-inoculants and elemental sulphur on Fe-P (mg/kg)

P fraction		**Fe-P**				**Fe-P**		
Stage		**60 DAS**				**110 DAS**		
FYM	**Phosphate**	**Solubilizing**	**Treatments**		**Phosphate**	**Solubilizing**	**Treatments**	
Levels	***Bacillus megaterium***	***Pseudomonas chlororaphis***	**Elemental sulphur**	**MEAN**	***Bacillus megaterium***	***Pseudomonas chlororaphis***	**Elemental sulphur**	**MEAN**
FYM @ 0%	19.3	18.9	18.6	18.9	18.6	17.9	17.7	18.0
FYM @	20.7	21.6	20.6	20.9	19.8	20.5	19.7	20.0

50%								
FYM @ 100%	22.0	22.1	19.8	21.3	21.2	21.3	18.8	20.4
FYM @ 150%	24.8	23.1	22.5	23.4	23.8	22.2	21.4	22.4
Mean	21.7	21.4	20.3	21.1	20.8	20.4	19.4	20.2
	FYM	PST	FYM×PST		FYM	PST	FYM×PST	
SED	0.294	0.255	0.510		0.205	0.177	0.355	
CD @ 0.05	0.608	0.526	NS		0.423	0.367	0.734	

Table 5: Effect of FYM, bio-inoculants and elemental sulphur on Al-P (mg/kg)

P fraction		**Al-P**				**Al-P**		
Stage		**60 DAS**				**110 DAS**		
FYM	**Phosphate**	**Solubilizing**	**Treatments**		**Phosphate**	**Solubilizing**	**Treatments**	
Levels	***Bacillus megaterium***	***Pseudomonas chlororaphis***	**Elemental sulphur**	**MEAN**	***Bacillus megaterium***	***Pseudomonas chlororaphis***	**Elemental sulphur**	**MEAN**
FYM @ 0%	15.9	14,9	14.5	15.1	14.3	14.0	13.5	13.9
FYM @ 50%	16.8	17.3	16.6	16.9	16.0	16.4	15.7	16.0
FYM @ 100%	17.6	17.9	16.1	17.2	16.7	16.9	15.2	16.2
FYM @ 150%	18.7	18.7	18.5	18.6	17.9	17.7	17.4	17.6
Mean	17.2	17.2	16.4	16.9	16.2	16.2	15.4	15.9
	FYM	PST	FYM×PST		FYM	PST	FYM×PST	
SED	0.191	0.166	0.332		0.209	0.181	0.362	
CD @ 0.05	0.396	0.343	0.686		0.431	0.373	NS	

Table 6: Effect of FYM, bio-inoculants and elemental sulphur on Ca-P (mg/kg)

P fraction		**Ca-P**				**Ca-P**		
Stage		**60 DAS**				**110 DAS**		
FYM	**Phosphate**	**Solubilizing**	**Treatments**		**Phosphate**	**Solubilizing**	**Treatments**	
Levels	***Bacillus megaterium***	***Pseudomonas chlororaphis***	**Elemental sulphur**	**MEAN**	***Bacillus megaterium***	***Pseudomonas chlororaphis***	**Elemental sulphur**	**MEAN**
FYM @ 0%	69.7	65.5	62.6	65.9	63.5	62.3	58.9	61.5
FYM @ 50%	76.7	76.8	75.4	76.3	72.4	77.9	73.9	74.7
FYM @ 100%	86.0	91.5	71.3	82.9	82.1	88.5	67.2	79.2
FYM @ 150%	100.4	99	95.3	98.2	98.2	96.1	93.0	95.7
Mean	83.2	83.2	76.1	80.8	79.0	81.2	73.2	77.8
	FYM	PST	FYM×PST		FYM	PST	FYM×PST	
SED	0.928	0.804	1.60		1.12	0.97	1.94	
CD @ 0.05	1.91	1.65	3.31		2.32	2.01	4.02	

Table 7: Effect of FYM, bio-inoculants and elemental sulphur on Available P (kg/ha)

P fraction		**Available P**				**Available P**		
Stage		**60 DAS**				**110 DAS**		
FYM	**Phosphate**	**Solubilizing**	**Treatments**		**Phosphate**	**Solubilizing**	**Treatments**	
Levels	***Bacillus megaterium***	***Pseudomonas chlororaphis***	**Elemental sulphur**	**MEAN**	***Bacillus megaterium***	***Pseudomonas chlororaphis***	**Elemental sulphur**	**MEAN**
FYM @ 0%	21.9	21.4	21.1	21.4	20.9	20.3	20.0	20.4
FYM @ 50%	22.7	23.0	22.3	22.6	21.6	21.9	21.5	21.6
FYM @ 100%	23.2	23.2	22.1	22.8	22.0	22.5	21.0	21.8
FYM @ 150%	25.3	24.7	24.4	24.8	24.1	23.8	23.5	23.8
Mean	23.2	23.1	22.4	22.9	22.1	22.1	21.5	21.9
	FYM	PST	FYM×PST		FYM	PST	FYM×PST	
SED	0.269	0.233	0.466		0.234	0.203	0.406	
CD @ 0.05	0.555	0.481	NS		0.484	0.419	NS	

Among the PSTs, *Bacillus megaterium* and *Pseudomonas chlororaphis* performed on par with each other in registering Al-P (17.2 and 16.2mg/kg in each stage respectively) and better in increasing the Al-P fractions in soil, when compared to that of elemental sulphur in both the stages. Phosphate solubilising microorganisms solubilize inorganic phosphate (including soil phosphate) with the production of inorganic (carbonic and sulfuric) and organic (citric, butyric, oxalic, malonic, lactic and etc.) acids and phosphatase enzyme (Sundara *et al*., 2001) [25] which might have helped to improve the inorganic fractions in soil.

The interactive effect of PSTs with FYM has significant effect on Al-P fractions at tasseling stage of the crop. The PSTs interacted with FYM at 150 percent are on par with each other (18.7, 18.7 and 18.5mg/kg respectively) followed by other treatments. The lowest values were recorded for the treatments involving PSTs without FYM.

Ca-P

Among the P fractions, Ca-P was the dominant form. The Ca-P status in the soil varied from 62.6mg/kg to 100.4 mg/kg at tasseling stage and 58.9 to 98.2mg/kg at the time of harvest (Table 6). In both the stages, among the FYM levels, the highest Ca-P fraction was recorded in the treatment with 150 percent of FYM (98.2 and 95.7mg/kg respectively) and lowest in treatment without FYM. Due to calcareous nature of the experimental soil, the Ca-P fraction dominated among all other inorganic P fractions (Kothandaraman and Krishnamoorthy, 1977) [9]. Increasing rate of FYM addition resulted in the concomitant increase in the Ca-P of the soil due to high concentration of soil Ca that might have reacted with the Pi in the FYM (Singaram and Kothandaraman, 1993)[21]. Addition of FYM has enhanced the microbial activity and thereby the production of organic acids on the decomposition of organic matter resulted in the formation of Ca-P. Addition of manure to soil raised phosphorus concentrations associated with calcium was also supported by Yin and Liang (2013) [33].

Among the PSTs, *Bacillus megaterium* and *Pseudomonas chlororaphis* performed on par with each other (83.2 and 83.2mg/kg respectively) at tasseling and better in increasing the Ca-P fractions in soil, when compared to that of elemental sulphur. The increase in Ca-P fraction by those Phosphate solubilising bacteria might be due to the fact that production of organic acids would have helped release of P from organic sources and helped in the formation of inorganic fraction (Saied *et al.,* 2018) [19]. At the harvest stage, the performance of *Pseudomonas chlororaphis* established higher (81.2mg/kg) than that of *Bacillus megaterium* (79.0mg/kg) and elemental sulphur.

Interactive effect of PSTs with FYM on Ca-P fractions has significant effect on the Ca-P fractions at both tasseling and harvest stage of the crop. The highest values were recorded for the interactive effect of *Bacillus megaterium* and *Pseudomonas chlororaphis* with FYM at 150 percent respectively (100.4 and 99.0mg/kg respectively at tasseling and 98.2 and 96.1mg/kg respectively at harvest) followed by the interactive effect of elemental sulphur with FYM at 150 percent. The lowest values were recorded for the interactive effect of PSTs without FYM.

Available P

The available P in the soil ranged from 21.1 to 25.3kg/ha at tasseling stage and 20.0 to 24.1 kg/ha at the harvest stage of the crop due to various treatments. Among the levels of FYM, the highest available P value was obtained in the treatment with FYM at 150 percent (24.8kg/ha) followed by other treatments in both the stages. The FYM at 100 percent and 50 percent are on par with each other. The lowest available P was obtained in the treatment without FYM. Addition of manure would have improved the microbial activity which in turn might have led to release of P from decomposing organic sources as well as from organic P. It is also in conformity with the findings of Verma *et al*., (2005) [30] who found release of phosphorus from organic matter during the mineralization process. It was also supported by Badawy *et al*., (2011) [1] who found that the available P in calcareous soils of Egypt increased from 11.91 to 16.68mg/kg with the increased application of FYM. Picolo and Huluka (1986) [15] reported that there is highly positive correlation between organic P and available P due to the mineralization of organic matter.

Among the PSTs, *Bacillus megaterium* and *Pseudomonas chlororaphis* performed on par with each other (23.2 and 23.1kg/ha respectively at tasseling and 22.1 and 22.1kg/ha respectively at harvest) and better in increasing the available P in soil, when compared to that of elemental sulphur in both the stages. The excretion of organic acids by the phosphor bacteria results in the acidification of the microbial cells and the surroundings, thereby P ions are released by substitution of H^+ for Ca^{2+} (Goldstein, 1994) [3]. The mineralization of organic P by the microbes too contributes for increase in the available P status of the soil as observed by Viswanath and Doddamani (1991) [31].

Conclusion

All the inorganic P fractions increased with the increasing level of FYM and were higher under the application of 150 percent of FYM. Among the PSTs, both *Bacillus megaterium* and *Pseudomonas chlororaphis* performed better than that of elemental sulphur. Both the bio-inoculants and FYM had significant effect on all the fractions individually but has no significant effect as a whole, except in case of Ca-P fractions. Compared to PSTs, the FYM at 150 percent showed significant effect on the transformation of inorganic P fractions. Among all the fractions, Ca-P fraction dominated due to calcareous nature of the experimental soil.

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