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RESEARCH PAPER

Impact of phosphorus levels and PSB dosages on soil microbial population and enzymatic activities in a vertisol

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Abstract:A pot culture experiment was carried out to study the impact of phosphorus levels and PSB dosages on soil microbial population and enzymatic activities in a *vertisol* during *kharif* 2018 at College of Agriculture, Raichur using maize as a test crop. A total of eleven treatments that includes different levels of phosphorus which are studied either alone or in combination with two levels of phosphate solubilizing bacteria (PSB). These treatments were replicated thrice and arranged in a completely randomized block design. The results revealed that soil application of PSB at 25 kg ha^{-1} along with complete dosage of NPK showed a significant effect on total bacterial count (2.67×10^9 g^{-1} of dry soil). Moreover, it also recorded the higher soil enzymatic activities *viz*., dehydrogenase activity (11.68 µg TPF $g^{-1}hr^{-1}$) and alkaline phosphatase activity (14.1 µg pNP $g^{-1}hr^{-1}$) and was at par with the treatment receiving 100 % NK + 75 % P + PSB @ 12.5 kg ha^{-1} and was superior to other treatments. Apart from these 100 % NK + 75 % P + PSB @ 12.5 kg ha^{-1} showed a significantly highest PSB count in soil (6.50×10^4 g^{-1} of dry soil). Lower soil microbial population and enzymatic activity were obtained in absolute control and thus indicated that application of inorganic phosphorus as well as treating the soil with PSB @25 kg ha^{-1} is beneficial in terms of higher microbial population and enzymatic activities in soil. A significant positive correlation was found between available P with plant growth parameters indicating that with the addition of PSB more P was solubilized and positively supported plant growth.

Key words: Dehydrogenase enzyme, Microbial, Phosphorus, Phosphatase enzyme

Introduction

Vertisols are characterized by high content of expansive clays that shrink and swell depending upon the prevailing soil moisture regime. They are estimated to occupy an area of 27 m ha in India and predominant in states of Madhya Pradesh (10.7 m ha), Maharashtra (5.6 m ha) and Karnataka (1.8 m ha). Vertisols are dark-coloured soils with moderate humus content and may also be characterized by salinity and well-defined layers of calcium carbonate or gypsum. In general, majority of the vertisols found in arid and semi-arid climate are calcareous. These soils are generally fertile soils but have the productivity constraints due to higher P fixation and immobilization of micronutrients. The P applied to calcareous vertisols through mineral fertilizers is ûxed soon and most of it becomes unavailable. A substantial amount precipitate as $[Ca_3(PO_4)_2]$ and $[Mg_3(PO_4)_2]$ was observed. Consequently, the farmers in irrigation command areas and those practicing intensive agriculture are compelled to use higher doses of P fertilizer recurrently at a huge cost and labour, which is an environmentally detrimental practice. It is believed that soils that have witnessed large quantity of phosphatic fertilizers are enriched with precipitated P compounds in sub surface layers which is insoluble and unavailable to crops. However, these unavailable P compounds can be made available for the plant by treating the soils with phosphate solubilizing bacteria (PSB). The growth and colonization of PSB is influenced by chemical, physical and biological properties of the soil. The availability of macro and micro nutrient elements can limit microbial population growth in a particular soil ecosystem. Essential soil elements for plant growth, such as, nitrogen, phosphorus, potassium, sulphur and micro nutrients influences the PSB population as these nutrient elements are also needed for microbial growth and activity (Naher *et al*., 2013). The active strains of PSB involved in this conversion are *Pseudomonas*, *Mycobacterium, Micrococcus, Bacillus, Flavobacterium, Rhizobium, Mesorhizobium* and *Sinorhizobium* (Rodriguez and Fraga, 1999). PSB have been reported to modify P nutrition and increase its solubilization in soil through many processes such as, they may decrease the pH of the soil by the producing organic (gluconic acid) and mineral acids, alkaline phosphatases, phytohormones and H^+ protonation, anion exchange, chelation and siderophores production which promote P solubilization in soil (Gyaneshwar *et al*., 2002). The soil microbial community reacts to external stress even more than plants and animals (Panikov, 1999). Hence, the present study was undertaken to study the impact of phosphorus levels and PSB dosages on soil microbial population and enzymatic activities in a vertisol using maize as a test crop.

Material and methods

A pot culture experiment on "Impact of phosphorus levels and PSB dosages on soil microbial population and enzymatic activities in a *vertisol*" was carried out in a glasshouse during *kharif*, 2018 at College of Agriculture, Raichur using maize as a test crop. The soil was deep black with clay loam texture having slight alkaline pH (7.72) and low EC (0.28 dS m^{-1}) and low in soil organic carbon content (4.90 g kg^{-1}). On the other hand, the soil was medium in available nitrogen (282.8 kg ha^{-1}), available phosphorous (21.27 kg ha^{-1}) and high in potassium (321.2 kg ha^{-1}). Treatments were arranged in a completely randomized block design, with eleven treatments each was replicated thrice. The treatment details are T_1: Absolute control; T_2: PSB (@12.5 kg ha^{-1}); T_3 100 % NPK (No PSB treatment) ; T_4 : 100 % NPK + PSB (@ 12.5 kg ha^{-1}); T_5 : 100 % NPK + PSB (@ 25.0 kg ha^{-1}) ; T_6 : 100 % NK + 75 % P (No PSB treatment); T_7: 100 % NK + 75 % P + PSB (@ 12.5 kg ha^{-1}); T_8 : 100 % NK + 75 % P + PSB (@ 25.0 kg ha^{-1}) ; T_9 100 % NK + 50 % P (No PSB treatment) ; T_{10}: 100 % NK + 50 % P + PSB (@ 12.5 kg ha^{-1}) and T_{11}: 100 % NK + 50 % P + PSB (@ 12.5 kg ha^{-1}).

Experimental pots were applied with PSB, a night before sowing of seeds according to PSB treatments. The PSB strain and the maize variety taken for the experiment was *Pseudomonas sp* and NK-6240 respectively. The recommended dose of fertilizer is 150 kg N, 65 kg P_2O_5 and 65 kg K_2O ha^{-1}. Phosphorus was applied at different levels according to treatments through SSP before sowing of the seeds. About 4-5 seeds were sown and two healthy seedlings were maintained up to 60 DAS till uprooting of test crop and soil samples were taken for microbial analysis.

Soil microbial analysis

Soils samples taken from experimental pots were kept in refrigerator. The fresh samples were used for determination of total plate count and PSB count by the procedure serial dilution technique method by Pramer and Schmidt (1964) and Rao and Sinha (1963). Phosphatase activity (PHA) was determined in fresh samples as per the procedure described by Tabatabai and Bremner (1969) and dehydrogenase activity (PHA) was determined in fresh samples as per the procedure described by Casida *et al*. (1965).

Statistical analysis of data

The experimental data obtained were subjected to statistical analysis using Fisher's method of analysis of variance as out lined by Panse and Sukhatme (1985). The level of significance used in 'F' and 't' tests was P = 0.01.

Results and discussion

Dehydrogenase activity

The dehydrogenase activity was found to be significantly highest in treatment that received 100 % NPK + PSB @ 25 kg ha^{-1} (11.68 µg TPF g^{-1} hr^{-1}) and was found to be at par with treatment receiving 100 % NK + 75 % P + PSB @ 12.5 kg ha^{-1} (11.39 µg TPF g^{-1} hr^{-1}) (Table 1). The lowest dehydrogenase activity was obtained in treatment T_1: Absolute control (6.07 µg TPF g^{-1} hr^{-1}).

Dehydrogenase activity is commonly used as an indicator of biological activity in soil (Duarah *et al.,* 2011) and was observed significantly higher in treatment that received complete dosage of fertilizers along with PSB. This could be due to the fact that dehydrogenase activity reflects the total oxidative activity of viable soil microflora and is directly related to the change in microbial biomass and activities as a result of higher dosage of application of PSB. As a consequence of greater biological activity due to excess PSB application and the presence of complete dosage of inorganic fertilizers favoured highest dehydrogenase activity (Khilpa *et al*., 2017). These results were also in accordance with literatures of Aseri *et al*. (2008) and Adak *et al*. (2014).

Table 1. Effect of various treatments on enzymatic activities in soil samples at 60 DAS

Treatments	Dehydrogenase activity (µg TPF $g^{-1}hr^{-1}$) 60 DAS	Phosphatase activity(µg pNP $g^{-1}hr^{-1}$) 60 DAS
T_1	6.07	8.02
T_2	8.43	8.30
T_3	8.57	12.50
T_4	11.07	13.23
T_5	11.68	14.10
T_6	8.59	9.90
T_7	11.39	13.92
T_8	10.23	12.60
T_9	8.61	8.61
T_{10}	9.61	8.71
T_{11}	9.60	9.60
S.Em.±	0.10	0.11
C.D. at 1%	0.41	0.46

Note: T_1: Absolute control, T_2: T_1+ PSB (@ 12.5 kg ha^{-1}), T_3: 100 % NPK (No PSB treatment), T_4: 100 % NPK + PSB (@ 12.5 kg ha^{-1}), T_5: 100 % NPK + PSB (@ 25.0 kg ha^{-1}), T_6: 100 % NK + 75 % P (No PSB treatment), T_7: 100 % NK + 75 % P + PSB (@ 12.5 kg ha^{-1}), T_8: 100 % NK + 75 % P + PSB (@ 25.0 kg ha^{-1}), T_9: 100 % NK + 50 % P (No PSB treatment), T_{10}: 100 % NK + 50 % P + PSB (@ 12.5 kg ha^{-1}), T_{11}: 100 % NK + 50 % P + PSB (@ 25.0 kg ha^{-1}).

Alkaline phosphatase activity

Significantly higher phosphatase activity was observed in a treatment that received complete NPK fertilizer along with excess dosage of PSB (14.1 µg pNP g^{-1} hr^{-1}) and it was found to be on par with treatment receiving NPK fertilizer and PSB at 12.5 kg ha^{-1} (13.92 µg pNP g^{-1} hr^{-1}) (Table 1). The lowest alkaline phosphatase activity was obtained in treatment T_1: Absolute control (8.02 µg pNP g^{-1} hr^{-1}).

Significantly higher phosphatase activity was observed in a treatment that received complete dosage of NK with three fourth of P fertilizer along with recommended dosage of PSB. This would be due to availability of more amount of phosphorus in the medium and ability of PSB to solubilize it (Barik and Purushothaman, 1998). It was also observed that there was a positive correlation between phosphate solubilizing capacity and alkaline phosphatase activity. The results of other workers also correlates with this study (Ponmorugan and Gopi, 2006).

Total bacterial population

The observations on the population of bacteria at 60 DAS was significantly higher in treatment receiving complete dosage of fertilizers along with PSB at 25 kg ha^{-1} (2.67 × 10^9 g^{-1} of dry soil) (Table 2), which was found to be at par with all the treatments receiving PSB and different levels of P (2.33 and 2.17 × 10^9 g^{-1} of dry soil). The significantly lowest bacterial population (1.5 × 10^9 g^{-1} of dry soil) was obtained in uninoculated absolute control treatment T_1.

Significantly highest total bacterial count was observed in a treatment that received complete dosage of inorganic fertilizers along with PSB. These readings are in similar with findings of Talwar *et al*. (2017) and Stephen *et al*. (2015).

Total PSB population

The PSB population was found to be significantly highest in treatment that received 100 % NK + 75 % P + PSB @ 12.5 kg ha^{-1} (6.50 × 10^4 g^{-1} of dry soil) fallowed by treatment receiving complete dosage of fertilizers along with PSB at 25 kg ha^{-1} and 12.5 kg ha^{-1} (6.33 and 6.17 × 10^4 g^{-1} of dry soil) (Table 2). However, these treatments were found on par to significantly superior treatment. The significant lowest PSB population (3.17 × 10^4 g^{-1} of dry soil) was recorded in uninoculated absolute control treatment T_1.

Significantly higher PSB population was obtained in treatment that received NPK fertilizers along with recommended dosage of PSB. This could be due to presence excess P solubilizers and also successful adaptation and proliferation of introduced PSB into rhizosphere soil of experimental pots and thus well established. These readings are in similar with findings of Stephen *et al*. (2015).

Correlation coefficient between available P, plant height, number of leaves, LA, LAI, dry matter plant^{-1} and root length

There was significant positive correlation found between available P with plant growth parameters *viz*., plant height, number of leaves, LA, LAI, dry matter plant^{-1} and root length (Table 3). The available P (Olsen's P) was positively correlated with plant height and root length and also positively significantly correlated with number of leaves at 5 % level of significance. Moreover, it was positively significantly correlated with LA, LAI and dry matter plant^{-1} at 1 % level of significance. These findings are in collaboration with the findings of Panhwar *et al.* (2011) who observed positive significant effect owing to phosphorus application rates.

Table 2. Effect of various treatments on microbial population in soil samples at 60 DAS

Treatments	Total Bacterial count (cfu × 10^9 g^{-1} dry soil) 60 DAS	PSB count (cfu × 10^4 g^{-1} dry soil) 60 DAS
T_1	1.50	3.17
T_2	1.67	3.33
T_3	1.83	5.33
T_4	2.17	6.17
T_5	2.67	6.33
T_6	1.83	4.33
T_7	2.33	6.50
T_8	2.33	5.83
T_9	1.67	3.83
T_{10}	2.17	4.00
T_{11}	2.33	4.33
S.Em.±	0.20	0.21
C.D. at 1%	0.80	0.85

Note: T_1: Absolute control, T_2: T_1+ PSB (@ 12.5 kg ha^{-1}), T_3: 100 % NPK (No PSB treatment), T_4: 100 % NPK + PSB (@ 12.5 kg ha^{-1}), T_5: 100 % NPK + PSB (@ 25.0 kg ha^{-1}), T_6: 100 % NK + 75 % P (No PSB treatment), T_7: 100 % NK + 75 % P + PSB (@ 12.5 kg ha^{-1}), T_8: 100 % NK + 75 % P + PSB (@ 25.0 kg ha^{-1}), T_9: 100 % NK + 50 % P (No PSB treatment), T_{10}: 100 % NK + 50 % P + PSB (@ 12.5 kg ha^{-1}), T_{11}: 100 % NK + 50 % P + PSB (@ 25.0 kg ha^{-1}).

Conclusion

Higher microbial populations followed by highest enzyme activities have been evident in the present study with the application of higher dose of PSB *i.e.,* 25 kg ha^{-1} along with recommended dosage of NPK fertilizers. These ûndings suggest that there is a strong relationship between PSB application and soil microbial population. PSB applications also increased the dehydrogenase and alkaline phosphatase activities. Further, a significant positive correlation was found between available P with plant growth parameters indicating that with the addition of PSB more P was solubilized and positively affected plant growth. Keeping these observations it can be concluded that application of higher doses of PSB in soils which are enriched with higher fixed phosphorus may be beneficial in reutilizing back for subsequent crop production.

Table 3. Correlation coefficient between Available P, Plant height, Number of leaves, LA, LAI, Dry weight and root length

	Available P	Plant height	Number of leaves	LA	LAI	Dry weight	Root length
Available P	_	0.594 0.053	0.669* 0.024	0.787** 0.004	0.767** 0.006	0.768** 0.006	0.595 0.054
Plant height		_	0.931** 0	0.910** 0.0001	0.905** 0.0001	0.865** 0.0006	1.00** 0
Number of leaves			_	0.894** 0	0.879** 0.0004	0.921** 0	0.931** 0
LA				_	0.997** 0	0.918** 0	0.909** 0.0001
LAI					_	0.914**	0.905** 0 0.00013
Dry weight						_	0.865** 0
Root length							_

Significance level are * = 0.05 and ** = 0.01

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