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Microbial Diversity in Various Combinations of Phosphorous Sources in Maize-Groundnut Cropping Sequence in an *Alfisols* of Odisha, India

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Low grade Rock phosphate (RP), Single super phosphate (SSP), Lime@0.2LR, Lime requirement (LR), Loamy acidic soil, Maize-Groundnut crop, Urea, Murate of potash (MOP), Lime requirement (LR), Pikovskaya medium, Colony Forming Unit (c.f.u.)

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A B S T R A C T

A field experiment was conducted with maize-groundnut cropping sequence. Rock phosphate and its combination with SSP were used as nutrient sources during Rabi 2016-17 (maize taken up in Kharif 2016). The study was carried out in the Central Farm, OUAT with the help of a field experiment laid out in Randomized Block Design with seven treatment T1 Control, T2 100% P(RP),T3 100% P(SSP), T4 75% P(RP) + 25% P(SSP), T5 50% P(RP) + 50% P(SSP), T6 25% P(RP) + 75% P(SSP) and T7 100% P(SSP) + Lime @ 0.2 LR and replicated in thrice. The soil of the experimental field was loamy acidic (pH 5.2) having Bray's P of 15.68 kg ha^{-1}. The different combinations with SSP were evaluated for their effectiveness in the cropping system. In addition to P applied @ 50kg P_2O_5 ha^{-1} and 40 kg P_2O_5 ha^{-1} to maize and groundnut crops respectively from various sources, N was added @ 150kgha^{-1} to maize and 20 kgha^{-1} to groundnut crop in the form of urea and K @50 and 40 kg K_2O ha^{-1} was added to maize and groundnut crop in the form of MOP. Highest maize grain yield (5.03 t ha^{-1}) was produced due to addition of 100%P (SSP) +Lime @ 0.2LR and highest pod yield (2.77 t ha^{-1}) of groundnut was also due to 100%P (SSP)+Lime@0.2LR. The total heterotrophic bacteria population (c.f.u. X 10^4 g^{-1}soil) was the highest in 100%P (SSP) + Lime@0.2LR in all the growth stage except flowering. Phosphorus solubilising bacteria population (c.f.u. X 10^4 g^{-1}soil) were maximum at pod formation stage in 100%P(SSP)+ Lime@0.2LR and 100% P(RP) treatment but at harvest the treatment received 100%P(SSP)+ Lime @0.2LR had the highest population.

Introduction

Phosphorus is regarded as the master "key" element in crop production because of its pivotal role in the normal growth and establishment of root system, Seed formation and harvesting of the crop maturity besides being an essential constituent of nucleic acids (Mangel and Kirkby, 1987). Phosphorus is one of the most essential major growth-limiting plant nutrient which affect the overall growth of plants (Wang *et al.*, 2009)[2] by influencing various key metabolic processes such as cell division and development, macromolecular biosynthesis, photosynthesis and respiration of plants (Shenoy and Kalagudi, 2005; Ahemad, 2009; Ahemad, 2012; Khan, 2009; Khan, 2013). The maximum part of soil

phosphorous, approximately 95-99% is present in the form of insoluble phosphates and hence it cannot be easily utilized by the plants (Kannapiran and SriRamkumar, 2011). Phosphorus is play an important role of biochemical storage and transfer of energy, cell elongation, root development, plant growth and several other processes in the living plant. Insoluble phosphate compounds like Rock phosphate can be solubilized by phosphatise enzymes produced by microorganisms in soil.

Insoluble phosphorous is solubilized by a major group of soil micro flora was reported and these complexes enabling plants to easily absorb phosphorous. Several reports have examined the ability of different bacterial species to solubilise insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate.

Phosphorus is considered as a limiting factor in plant nutrition due to the deficiency of available soluble phosphate in the soil. However, phosphobacterium, a phosphate solubilizing bacteria able to convert the unavailable phosphate present in the soil to an available form.

The use of phosphate solubilizing bacteria as inoculants simultaneously increase P uptake by plants (Rodriquez and Fraga, 1999), improve nodulation and hence increase symbiotic nitrogen fixation (Dametario *et al.,* 1972).

The effectiveness of rock phosphate of low reactivity can also be increased by application of rock phosphate to green manure crop preceding the main crop, inoculation of the field following rock phosphate application with either phosphate solubilizing micro-organism or mycorrhiza (Misra and Pattanayak, 1997).

Materials and Methods

The experimental site is located in the Central Farm, OUAT, Bhubaneswar which lies at 85^0 47’ 18” E latitude 20^0 16’ 51" N longitudes with an elevation of 25.9 meter above mean sea level. The summer months from March to May/ June are hot and humid. The mean minimum and maximum temperature were 22.6^{o}C and 32.6^{o}C respectively. Temperatures drop to approximately 15° C during these months. The physico-chemical properties of the soil of experimental site were loamy texture with pH 5.2 and Exchangeable Ca^{2+} 0.89 [cmol (p^+) kg^{-1}]. The soil had the available Bray’s P 15.68 kg ha^{-1} (medium), Available Nitrogen 239 kg ha^{-1} (low), Available Potassium 150 kg ha^{-1} (medium) and Organic carbon 3.4 g kg^{-1} Soil.

Experimental details

Field experimental design laid out in Randomized Block Design with seven treatment T1 Control, T2 100% P(RP),T3 100% P(SSP), T4 75% P(RP) + 25% P(SSP), T5 50% P(RP) + 50% P(SSP), T6 25% P(RP) + 75% P(SSP) and T7 100% P(SSP) + Lime @ 0.2 LR and replicated in thrice times.

Collection of soil sample

Fresh rhizospheric soil was collected from each treatment & replication at different growth stages to study the activity of soil microbial population (heterotrophic bacteria and Phosphorous solubulizing bacteria) by serial dilution and spread plate technique to ascertain the effect of different sources / combinations of P i.e. RP & SSP.

Methods enumeration of soil microbial population

The soil microbial population (heterotrophic bacteria and Phosphorous Solubilizing

bacteria) was determined by serial dilution and spread plate technique.

One (1) g of the collected soil samples were added to each of ten tubes containing 9ml distilled water thoroughly mixed and spread over petriplates containing Nutrient Agar medium and Pikovskaya medium for enumeration of total heterotrophic bacteria and Phosphorous Solubilizing Bacteria (PSB) population respectively.

The plates were incubated at 30°C for 24 hours for bacterial isolation and at 30°C for 48 hrs for heterotrophic bacteria and PSB population.

Calculation

$$\text{CFU / ml} = \frac{\text{No. of colony} \times \text{inverse of dilution taken}}{\text{vol. of inoculum taken}}$$

Results and Discussion

Total Heterotrophic Bacteria (c.f.u. X 10^4 g^{-1} soil)

It is clear that the population of heterotrophic Bacteria (c.f.u X $10^4 g^{-1}$soil) increased with the application of Phosphorous. It ranged from 44.0-78.67, 50.33-87.67and 54.67-76.67 (c.f.u X 10^4 g^{-1} soil) at all growth stages respectively. Among the various sources 100% P (SSP) + Lime @ 0.2 LR resulted in maximum population followed by 50% P (RP) + 50% P (SSP) at all growth stages.

The later was in turn followed by 100% P (Rock Phosphate) at all the cases. The steepness of change in the population was of the order of 100% P (SSP) + Lime @ 0.2 LR >50% P (RP) + 50% P (SSP) >100%P (RP)>75% P (RP) + 25% P (SSP)> 25% P (RP) + 75% P (SSP) >100% P (SSP).

Phosphorous solubilising bacteria (PSB) (c.f.u. X 10^4 g^{-1} soil)-

It is comprehensible from that the Phosphorous Solubilising bacteria (PSB) population (c.f.u X 10^4 g^{-1} soil) increased in all the treatments of applied Phosphorous. It ranged from 40.67-65.33, 44.00-75.00 and 47.33-65.33 (c.f.u. X 10^4 g^{-1}soil) at all groundnut stages respectively.

The treatment 100% P (SSP) + Lime @ 0.2 LR recorded the highest followed by 100% P (RP) phosphorous solubilising bacteria population at flowering and vice versa at pod formation. At groundnut harvest100% P (SSP) + Lime @ 0.2 LR recorded the highest followed by 75% P (RP) + 25% P (SSP).

At flowering the PSB population increased in the order 100 % SSP, 75% RP + 25% SSP, 50% RP + 50% SSP, 25% RP + 75% SSP, 100% P(SSP) + Lime 0.2 LR.

At pod formation the trend was 100 % SSP < 75% RP + 25% SSP <50% RP + 50% SSP<25% RP + 75% SSP<100%P(RP)< 100%P(SSP) + Lime@0.2LR.. At groundnut harvest the trend was 100 % RP<100% P(SSP)<50% RP + 50% SSP<25% P(RP) + 75% P(SSP) <75%P(RP)+25%P(SSP)<100% P(SSP)+Lime@0.2LR.

Total biomass production (t ha^{-1}) in the cropping sequence as affected by different P-sources

The total biomass production in maize which ranged from 5.59-11.15 t ha^{-1} with the treatment control and 100% P (SSP) +Lime@ 0.2 LR respectively. The highest biomass yield was produced by the treatment received 100% P (SSP) +Lime@ 0.2 LR (11.15 t ha^{-1}).

Crop Information and Inputs

Test Crop	Maize	Groundnut
Variety	PAC-752	TAG-24
Duration	120 days	120 days
Season	Kharif	Rabi
Fertilizer dose (N-P_2O_5-K_2O)kg/ha	150-50-50	20-40-40
Lime	@ 0.2 L.R	@ 0.2 L.R

Total Heterotrophic Bacteria (c.f.u. X 10^4 g^{-1} soil) at different growth stages of Groundnut crop treated with various P sources

Treatment		Harvest of Maize crop	Growth stage of Groundnut crop		
			Flowering	Pod formation	Harvest
T1	Control	48.33	44.00	50.33	54.67
T2	100% P(RP)	74.33	69.67	76.00	74.67
T3	100% P(SSP)	62.00	60.67	64.33	63.67
T4	75%P(RP)+25% P(SSP)	71.33	67.67	73.00	74.33
T5	50%P(RP)+50% P(SSP)	68.00	76.67	72.33	71.33
T6	25%P(RP)+75%P (SSP)	64.33	63.67	68.00	63.33
T7	100%P(SSP)+ Lime @0.2 L.R	89.67	78.67	87.67	76.67
	S.E.M(±)	2.63	2.29	1.97	2.27
	CD(0.05)	7.89	6.86	5.91	6.82

Total Heterotrophic Bacteria (c.f.u. X 10^4 g^{-1} soil)

Phosphorous solubilising bacteria (PSB) (c.f.u. X 10^4 g^{-1} soil) at different growth stages Groundnut crop treated with various P sources

Treatment		Harvest of Maize crop	Growth stage of Groundnut crop		
			Flowering	Pod formation	Harvest
T1	Control	46.67	40.67	44.00	47.33
T2	100% P(RP)	70.67	68.67	71.00	48.67
T3	100% P(SSP)	55.33	48.00	54.67	52.00
T4	75%P(RP)+25% P(SSP)	58.67	56.33	60.33	63.33
T5	50%P(RP)+50% P(SSP)	57.67	58.67	62.00	53.33
T6	25%P(RP)+75%P (SSP)	61.33	60.67	64.67	60.67
T7	100%P(SSP)+ Lime @0.2 L.R	70.67	72.67	75.00	65.33
	S.E.M(±)	1.96	2.22	2.55	2.46
	CD(0.05)	5.86	6.65	7.63	7.37

Phosphorous solubilising bacteria (PSB) (c.f.u. X 10^4 g^{-1}soil)

Total biomass production (t ha^{-1}) in the cropping sequence as affected by different P-sources

Treatment		Biomass production (t ha^{-1})						
		Maize			Groundnut			Maize+ Groundnut
		Grain	Stover	Total	Pod	Haulm	Total	Total biomass (t ha^{-1})
T1	Control	2.44	3.15	5.59	1.67	2.40	4.07	9.66
T2	100% P(RP)	4.13	5.21	9.34	2.47	3.37	5.83	15.17
T3	100% P(SSP)	4.35	5.80	10.15	2.23	3.80	6.03	16.18
T4	75%P (RP)+25% P(SSP)	4.90	5.97	10.87	2.50	3.87	6.37	17.24
T5	50%P (RP)+50% P(SSP)	4.94	5.83	10.77	2.60	4.73	7.33	18.10
T6	25%P (RP)+75%P (SSP)	4.30	4.90	9.20	2.10	3.50	5.60	14.80
T7	100%P (SSP)+ Lime@ 0.2LR	5.03	6.12	11.15	2.77	4.90	7.67	18.82
	S.E.M(±)	0.12	0.15	0.16	0.21	0.34	0.05	-
	CD(0.05)	0.35	0.44	0.49	0.62	1.02	0.14	-

The same trend was observed in total biomass production in groundnut which ranged from 4.07 – 7.67 t ha^{-1} the total biomass produced by cropping system ranged from 9.66-18.82 (t ha^{-1}) with control and 100% P (SSP) +Lime@ 0.2 LR. The treatment received 50% (RP) +50%SSP followed the 2nd highest.

The treatment 100%P (SSP) +Lime @0.2LR also recorded the highest yield, Biomass production, available Total Heterotrophic Bacteria population and Phosphorous solubilising bacteria population occurred as compared to other treatments.

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Abbreviations

P-Phosphorous
RP-Low grade Rock phosphate
SSP-Single super phosphate
LR-Lime requirement
c.f.u.-Colony Forming Unit

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