Bio-solubilization of Rock Phosphate and Plant Growth Promotion by *Aspergillus niger* TMPS1 in Ultisol and Vertisol

Manoj Shrivastava and S.F.D'Souza
Nuclear Agriculture and Biotechnology Division
Bhabha Atomic Research Centre Trombay,
Mumbai-400085
India

Main Concern Related to Phosphorus Research

- Agricultural point of view

  √ Phosphorus requirement for sustainable crop production

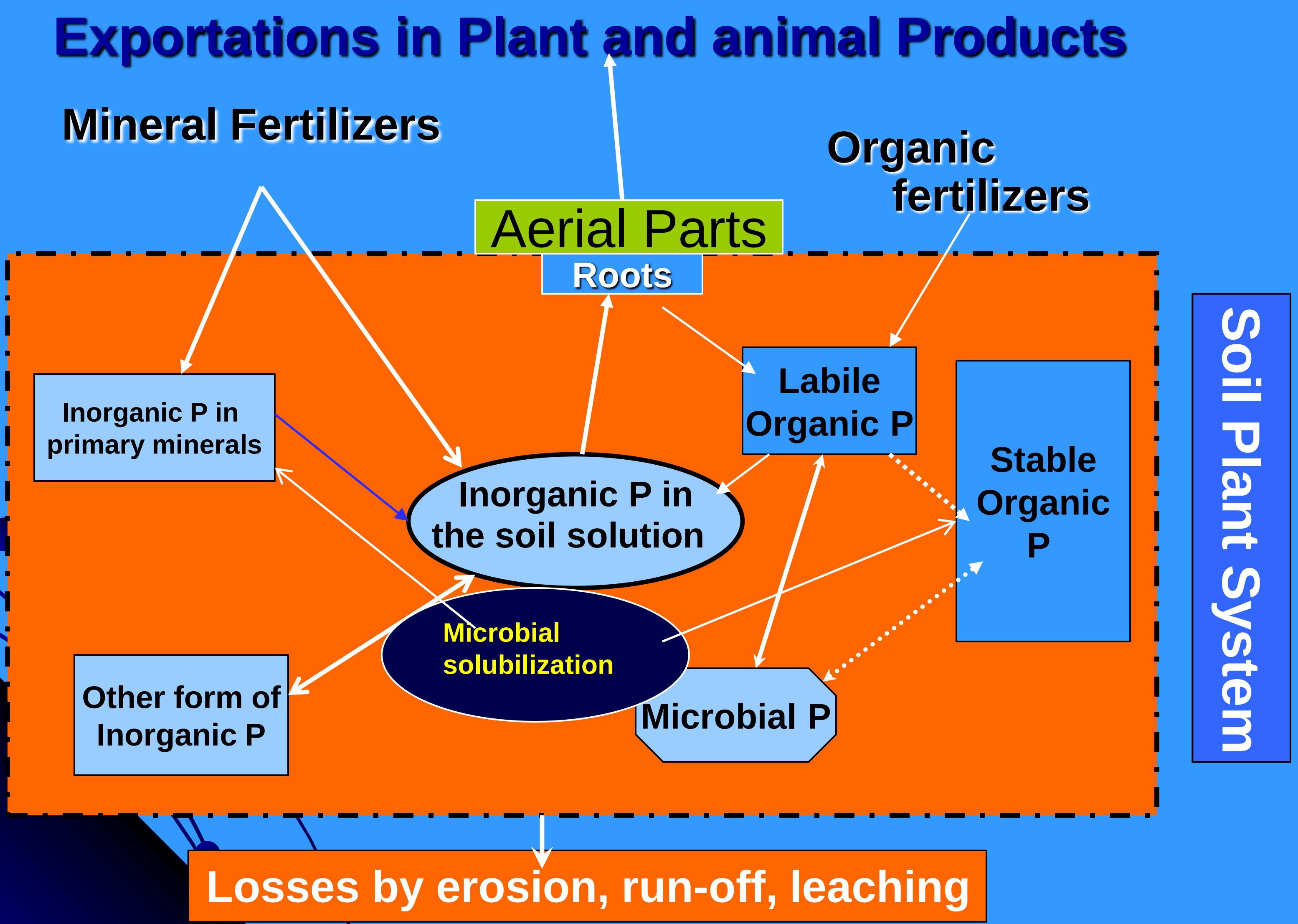
- Environmental point of view

  √ Soil or fertilizer phosphorus contribution to eutrophication of aquatic environments

Facts

- P is an essential plant nutrient
- Global Rock Phosphate (RPs) Production >160 mmt (2008)
- Most RPs are not suitable for phosphatic fertilizer production due to low reactivity and impurities present in them
- Low-grade rock phosphate can be utilized as direct application P fertilizer with or without processing
- Application of rock phosphate in conjunction with phosphate solubilizing microorganisms

Soil Phosphorus Cycle

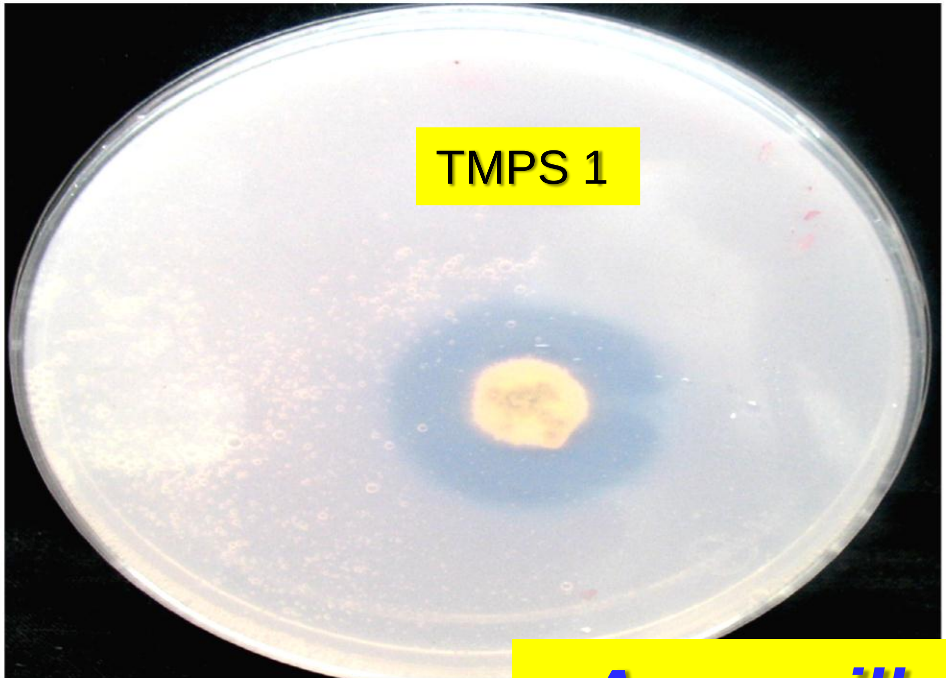


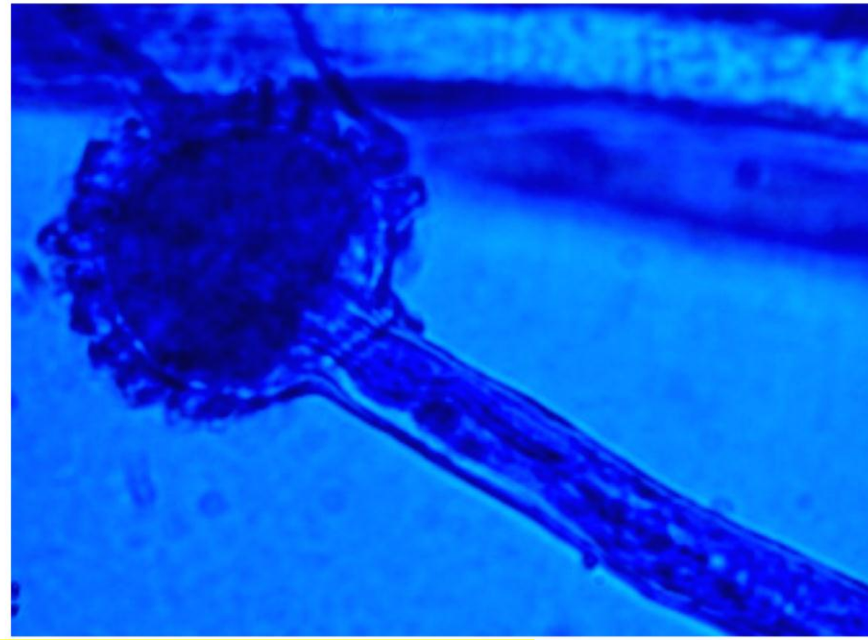
OBJECTIVES

The overall objective of this study was to evaluate the efficacy of a fungus *Aspergillus niger* strain TMPS1 isolated from mangrove rhizosphere as phosphate solubilizing bio-fertilizer under greenhouse conditions using ^{32}p isotope dilution technique

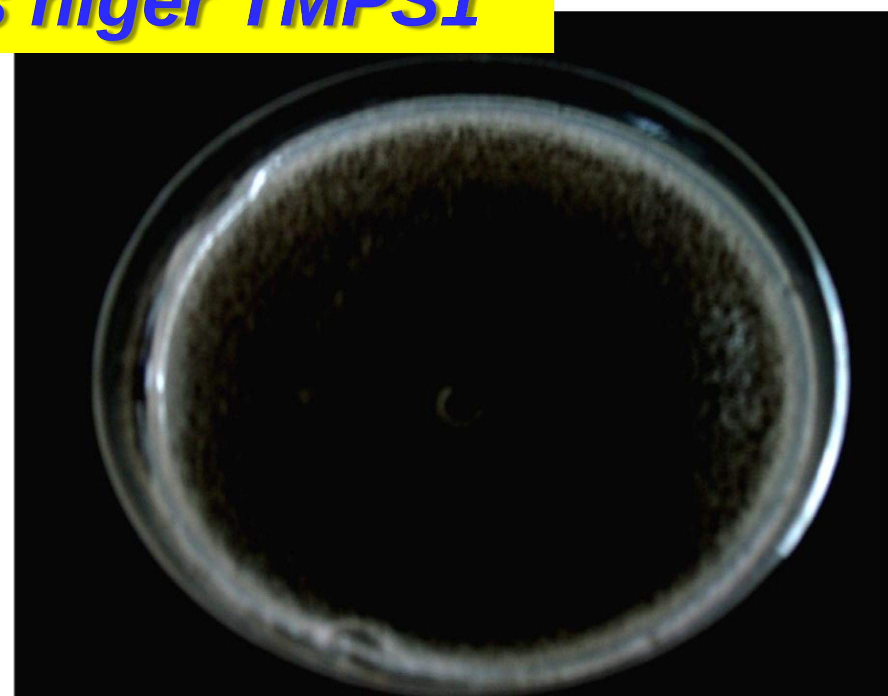
Isolation of Fungus from Soil

- Soil: Rhizospheric soil of Mangrove
- Media: Pikovskaya's agar plates
- Formation of a clear halo around the fungal growth after 5 days of incubation





Aspergillus niger TMPS1



Molecular Identification of Fungus

DNA Isolation

↓

Amplification of 18S rRNA gene

(NS5 [5’-AACTTAAAGGAATTGACGGAAG-3’] and NS8 [5’-CCGCAGGTTCACCTACGGA-3’])

↓

Cloning

pT257R/T

↓

18S rRNA sequencing and sequence analysis

M13 primer (TGTAAAACGACGGCCAGT)

Isolates	Length of ITS region Sequenced (bp)	Identification by ITS region sequencing	Gene identity (%)	GenBank Accession no.
TMPS1	**560**	***Aspergillus niger***	**98**	**DQ316605**

Radiotracers for phosphorus

- ^{32}P
- $T_{1/2}$----14.3 DAYS
- ß-EMITTER
- $E_{max.}$---1.71 MeV
- COUNTING

i) GM

ii) Cerenkov or LSC

- ^{33}P
- $T_{1/2}$----25 DAYS
- ß-EMITTER
- $E_{max.}$---0.248 MeV
- COUNTING

i) LSC

Greenhouse experimental details

- CROP — Wheat (*Triticum aestivum*)
- VARIETY — PbW 343
- SOIL — 2 kg soil per pot
  Black soil (Vertisol)
  Red Soil (Ultisol)
- TREATMENTS
  - Control (No P)
  - Soil+ ^{32}P
  - Soil+ TMPS1+ ^{32}P
  - Soil + LRP+^{32}P
  - Soil + LRP + TMPS 1+ ^{32}P
  - Soil + PRP + ^{32}P
  - Soil+ PRP+ TMPS 1 + ^{32}P
- EXPERIMENTAL DESIGN — CRD
- REPLICATIONS — 04
- Activity applied — 5 MBq ^{32}P kg^{-1} Soil

Characteristics of rock phosphates

Rock phosphate	Total P (%)	Citric Acid Soluble P (%)
Purulia (PRP)	14.4	1.623
Lalitpur (LRP)	9.8	0.425

PHYSICO-CHEMICAL CHARACTERISTICS OF EXPERIMENTAL SOIL

Sr. No.	Characteristics	Value	
		Vertisol	Ultisol
1	Texture	Clayey	Sandy Clay
2	pH (1:2) (soil:water)	8.20	4.8
3	Free $CaCO_3$ (g kg^{-1})	36	ND
4	0.05M $CaCl_2$ Extractable Al (mg kg^{-1})	ND	2.58
5	Moisture equivalent (%)	32.0	30.0
6	Organic carbon (g kg^{-1})	5.10	20.4
7	CEC (C mol (P+) kg^{-1})	51.13	8
8	Total N (g kg^{-1})	0.6	3.9
9	Available P (mg kg^{-1})	3.7 (Olsen's)	5.85 (Bray I)
10	P fixing capacity (g kg^{-1})	620	700

Parameters studied

- Dry matter yield -DMY (g pot^{-1})
- Total phosphorus uptake (mg pot^{-1})
- Specific Activity (Bq mgP^{-1})
- Phosphorus derived from lebeled soil (bioavailable P) and rock phosphate (mg pot^{-1})

CALCULATIONS

- Total P uptake by plant (mg P Pot^{-1}) U_{TP} = DMY (mg Pot^{-1}) X (% P/100)
- DMY- Dry matter yield of plant
- Specific Activity (Bq mg P^{-1}) = Bq g^{-1} plant/mg P g^{-1} plant

%PdfS -- Fraction of P in the plant derived from the soil

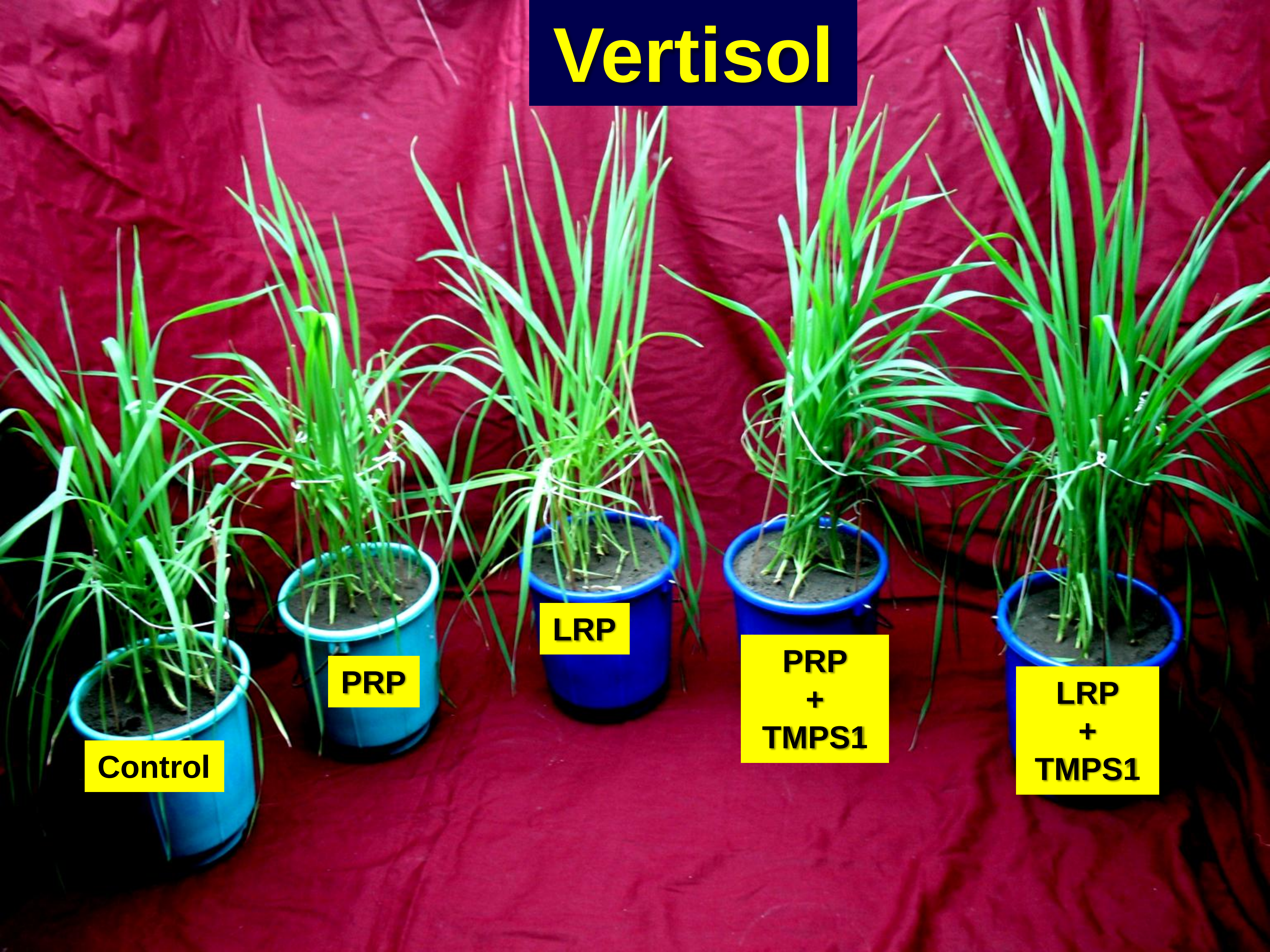
$$\% \text{ PdfS} = \frac{\text{SA in Plant (Bq/mg P) in presence of RP}}{\text{SA in plant (Bq/ mg P) in absence of RP}} \times 100$$

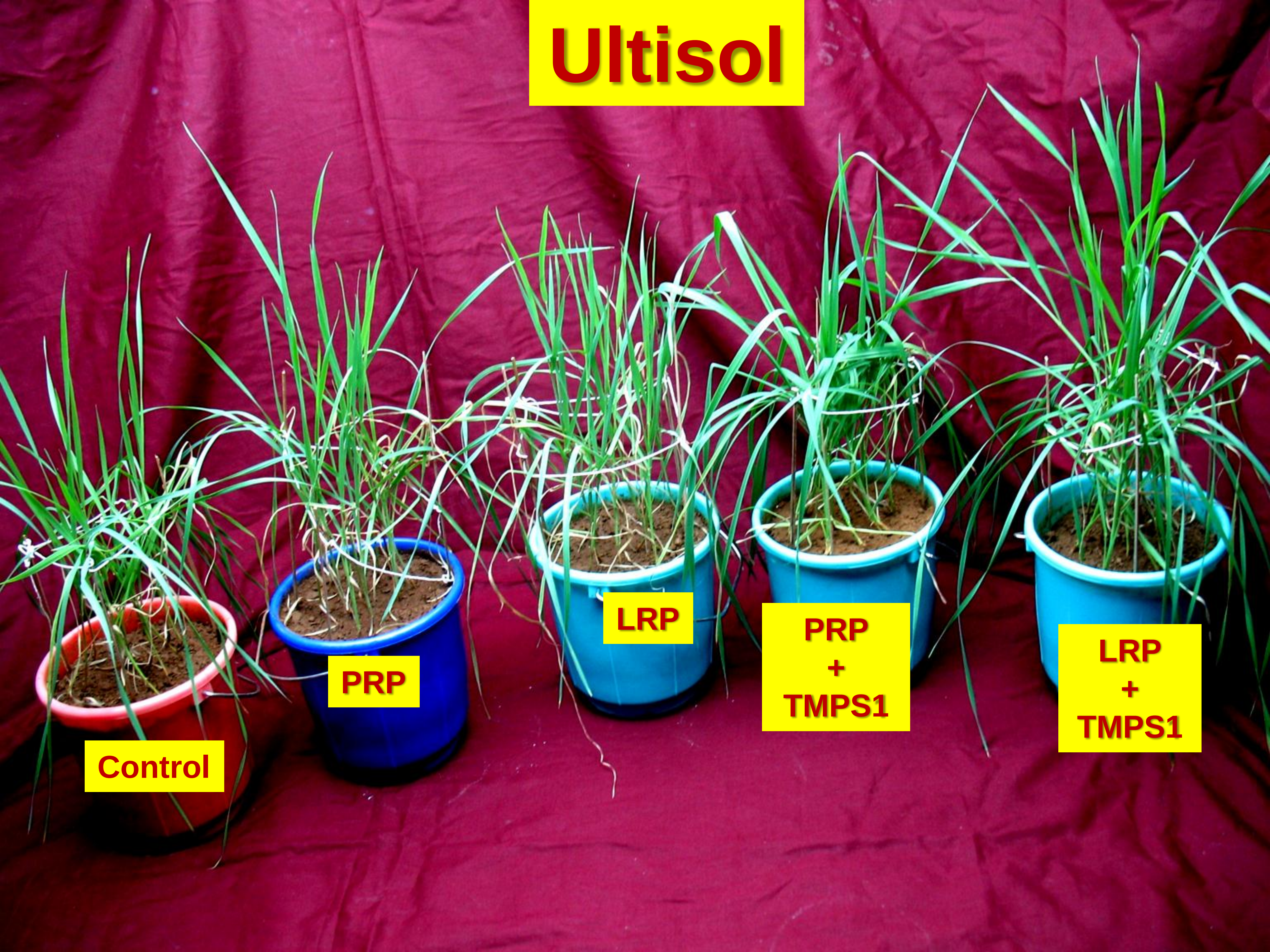
- %PdfRP----Fraction of P in the plant derived from the rock phosphate

$$\%\text{PdfRP} = 1 - \frac{\text{SA in Plant (Bq/mg P) in presence of RP}}{\text{SA in plant (Bq/ mg P) in absence of RP}} \times 100$$

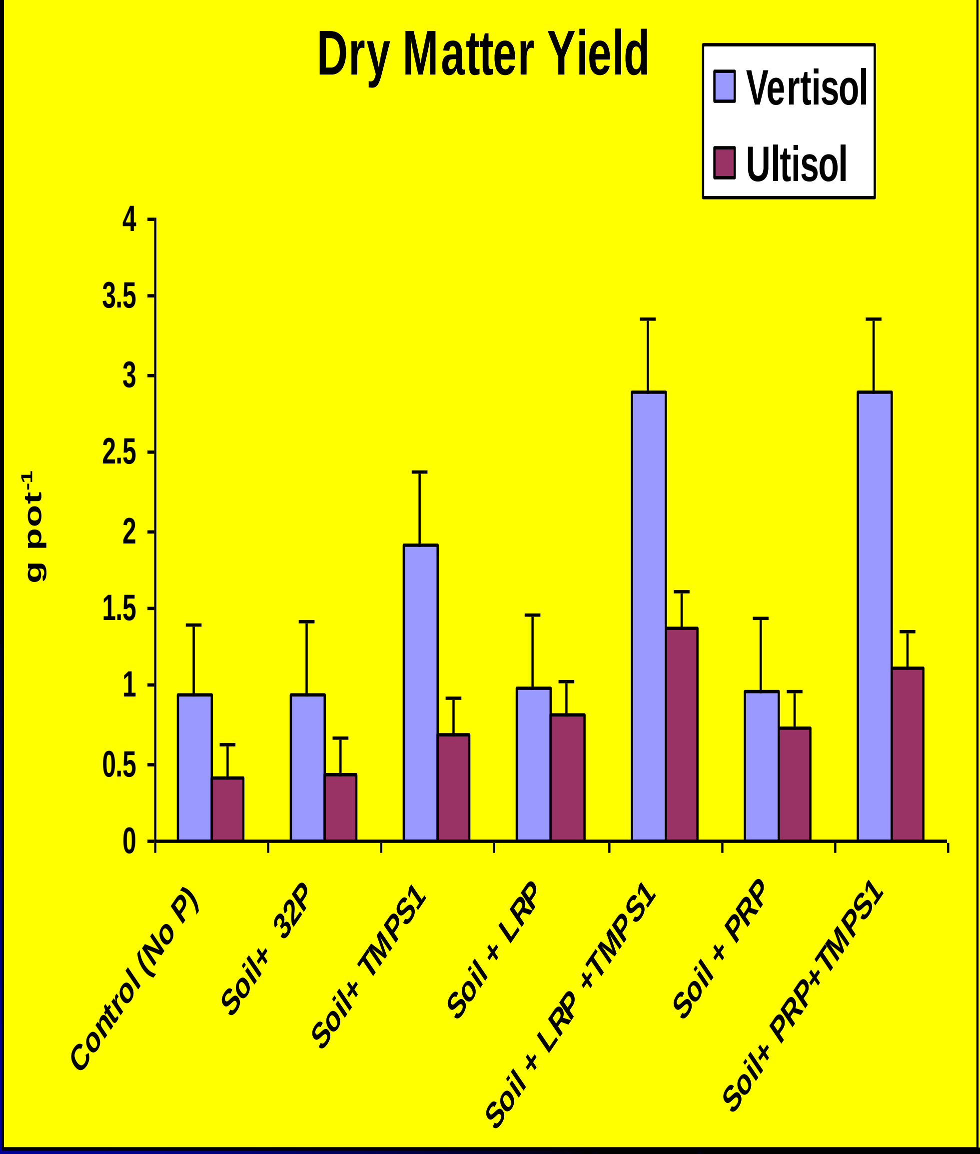
- P uptake from labeled source (mgP Pot^{-1}) = U_{TP} X (%PdfS/100)

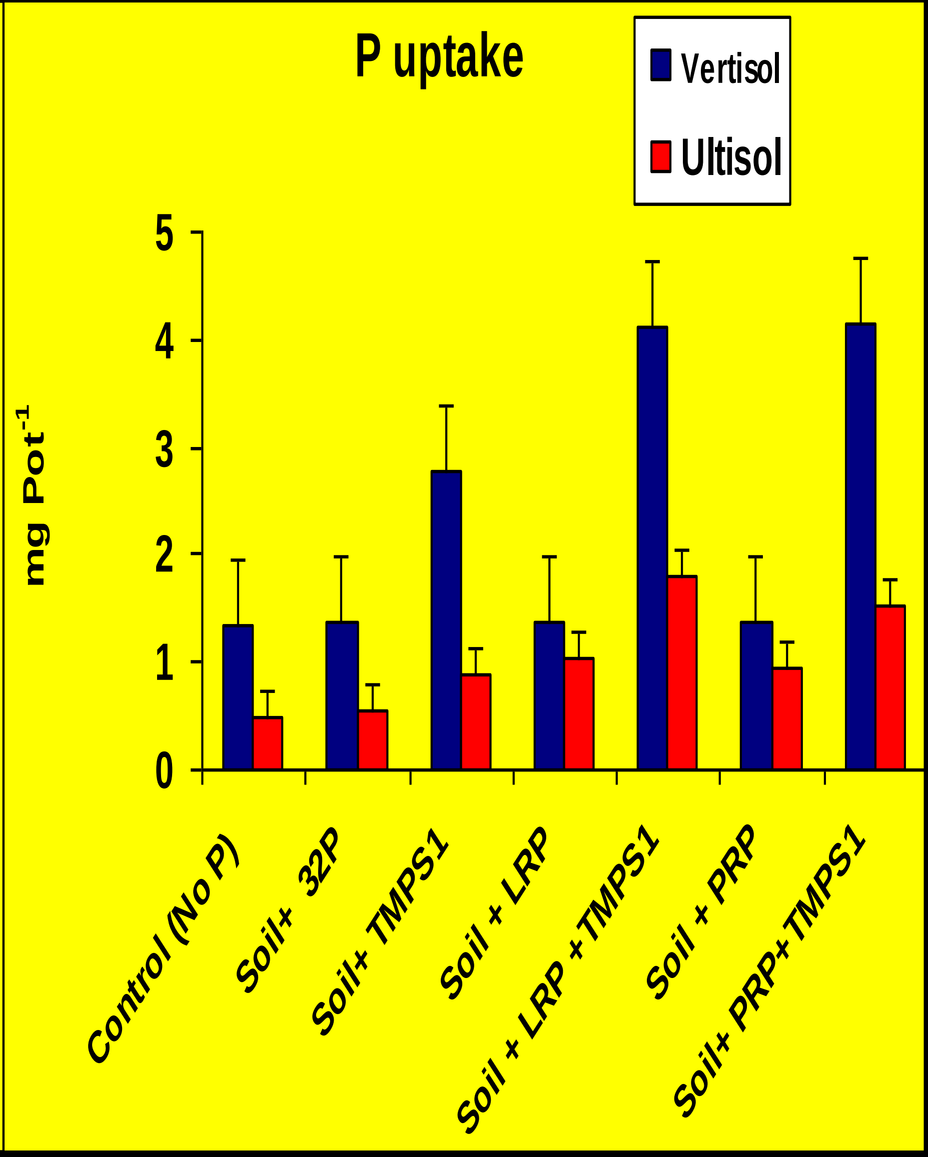
- P uptake from rock phosphate (mgP Pot^{-1}) = U_{TP} X (%PdfRP/100)


Vertisol
Control
PRP
LRP
PRP + TMPS1
LRP + TMPS1

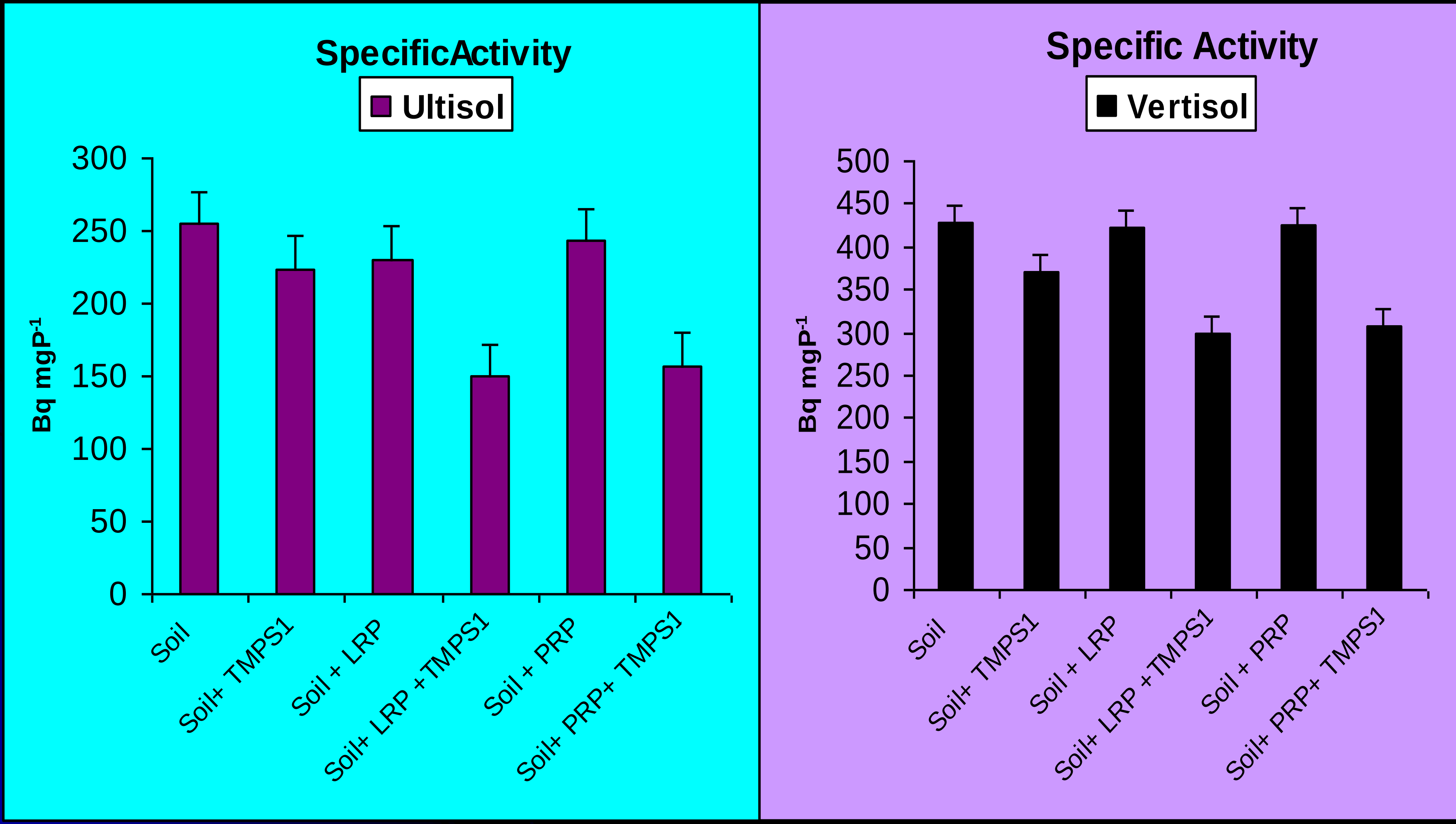

Ultisol
Control
PRP
LRP
PRP + TMPS1
LRP + TMPS1

Effect of fungus inoculation on shoot dry matter yield and P uptake of wheat in ultisol and vertisol





Effect of fungus inoculation on specific activity ($Bq\ mgP^{-1}$) of wheat in ultisol and vertisol



Effect of fungus inoculation on percentage of P derived from soil and rock phosphates in ultisol

Sr. No.	Treatments	Ultisol			
		PdfS		PdfRP	
		%	mgP pot^{-1}	%	mgP pot^{-1}
1	Soil + LRP+^{32}P	90.4	0.932	9.6	0.100
2	Soil + LRP + TMPS 1 +^{32}P	66.7	1.210	33.3	0.604
3	Soil + PRP + ^{32}P	95.3	0.906	4.7	0.045
4	Soil+ PRP+ TMPS1+^{32}P	70.3	1.063	29.7	0.449
LSD (P<0.05)			0.126		0.076

Effect of fungus inoculation on percentage of P derived from soil and rock phosphates in vertisol

Sr. No.	Treatments	Vertisol			
		PdfS		PdfRP	
		%	mgP pot^{-1}	%	mgP pot^{-1}
1	Soil + LRP+^{32}P	98.5	1.355	1.5	0.021
2	Soil + LRP + TMPS 1 +^{32}P	81.1	3.338	18.9	0.778
3	Soil + PRP + ^{32}P	99.3	1.356	0.7	0.010
4	Soil+ PRP+ TMPS1 +^{32}P	82.7	3.425	17.3	0.716
LSD ($P<0.05$)			0.825		0.095

Conclusions

- Potential use of *Aspergillus niger* TMPS1 isolate as a phosphate solubilizer in ultisol as well as vertisol.

- In general rock phosphates solubilization in alkaline vertisol does not occur, but this fungus solubilized native unavailable soil P as well as rock phosphate in vertisol.

- This study showed the advantages of using the ^{32}P isotope in distinguishing the contribution of bio-available native soil P and P from rock phosphates to P nutrition in plant – microbe interaction.

- This fungus should be evaluated as bio-fertilizer under field condition in different agro climatic regions for different crops.

Acknowledgement

- BRIT, DAE for providing ^{32}P radioisotope
- Head, NABTD and Director BMG, BARC, Mumbai, India

THANK-YOU