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IMPROVEMENT OF WHEAT GROWTH AND NUTRIENT UPTAKE BY PHOSPHATE SOLUBILISERS

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ABSTRACT
A pot experiment was carried out for evaluating the effect of P solubilising bacterial inoculants viz. 5*Bacillus*P, 5*Bacillus*M, 42*Bacillus*P, 42*Bacillus*M and 5*Bacillus*M+42*Bacillus*M in combinations along with different levels of SSP (30 mg $Kg^{-1}$and 60 mg Kg^{-1}) and URP (30 mg Kg^{-1} and 60 mg Kg^{-1}) on wheat growth. Stimulatory effects of 42*Bacillus*P, 42*Bacillus*M and 5*Bacillus*M+42*Bacillus*M in combination on population count, dry weight of shoot, total N and total P content were recorded at 30 mg Kg^{-1} SSP as compared to 60 mg Kg^{-1} SSP application. A significant correlation was observed between dry weight of shoot, total N and total P content at 70 days of sowing. The available P content of soil was also increased at 35 days of sowing while it decreased at 70 days of crop growth. The result suggests that 42 *Bacillus*M was able to dissolute more P and hence improved plant growth.

KEY WORDS: P solubilising bacteria, SSP, URP, Wheat.

INTRODUCTION

High yielding varieties are generally responsive to nutrient application. The fertilizer consumption was only 600g ha^{-1} in 1960-61 in Punjab and has gone upto 239.2 Kg ha^{-1} in 2012-13(IPNI, 2014). Similarly the consumption of pesticides, chemicals have also increased. Intensive agriculture requires heavy doses of chemicals. Moreover, it result in high input cost to farmers and due to this inherent financial strain they are unable to get sufficient returns. Various approaches have been employed to augment sustainable agriculture. Active approach is manipulation and management of biological system not only to maximize yields but to stabilize the agro system and to minimize the industrial input demands thus representing an integrated approach of the appropriate modern technology with traditional techniques. This has led to use of biofertilisers in combination with little chemical fertilizers and organic manures. It offers a great opportunity to increase crop production with less cost and saves environment. Phosphate solubilising microorganism (PSM) biofertiliser being economical and environmentally safe offers a viable alternative to chemical fertilizers. Among bacteria, aerobic spore forming bacteria are more effective P solubilisers. Phosphate solubilisation by these microorganisms is brought about by the production of organic acids some of which have been identified as malic, glyoxalic, succinic, fumaric, citric and Keto glutaric acid (Whitelaw, 1999; Panhwar *et al*., 2013). Addition of rock phosphate coupled to inoculation with PSM has given good response in many crops. The immobilized living cells can synthesize various useful chemicals using multienzyme reactions and regeneration activity to prolong their catalytic life (Tanaka and Nakajima, 1990). Phosphobacteria are better suited in neutral and alkaline soils and phosphofungi in acid soils (Gaind and Gaur., 1989; Chatli et al, 2008). The Punjab soils are alkaline in reaction. Therefore, the present study was undertaken with the objective of mass multiplication of PSB to see their effect under green house conditions taking wheat as a test crop.

MATERIALS & METHODS

Fifty Phosphate solubilising bacteria (PSB) isolated from rhizospheric soils of trans Himalayan region of Himachal Pradesh in association with *Hippophae rhamnoides*, *Salix alba* and *Robinia pseudoacacia* were tested for resistance to ten antibiotics viz. Streptomycin sulphate, Chloramphenicol, Vancomycin, Polymyxin, Erythromycin, Tetracycline, Ampicillin, Kanamycin, Gentamycin and Amoxycillin) at 50 µg ml^{-1} concentration. The screened microbes were further tested for their relative potentials to resist antibiotics at 100 µg ml^{-1}.

Quantitative estimation of P solubilisation in PVK and NBRIP broth

The selected antibiotic resistant microbes were used for testing their relative efficacies to solubilise insoluble inorganic sources of P viz. Tricalcium phosphate (TCP) and Udaipur rock phosphate (URP) in National Botanical Research Institute (NBRIP) broth (Nautiyal, 1999). 10^6 bacterial cells ml^{-1} were inoculated in 100 ml NBRIP broth and supplemented with one ml of each antibiotic in combination for which the screened organisms were showing resistance and incubated for 4 days under shake at 250 rpm. Uninoculated broth served as control.

Their P solubilisation was compared with their parents. The solubilised P-was determined in clear filtrate using Ascorbic acid method (Watanabe and Olsen, 1965). The intensity of blue colour was measured on spectrophotometer at 730 nm and the quantity of the solubilised P was expressed as µg ml^{-1}. The synergistic effect of these two mutants for P dissolution was also studied by inoculating them in NBRIP broth along with 100 µg ml^{-1} of Polymyxin and Erythromycin.

Estimation of organic acids in liquid media

The selected microbes were also tested for the production of organic acids by paper chromatography (Nordmann and Nordmann, 1960).

Soil sampling and physico-chemical properties

Soil low in available P was collected from Punjab Agricultural University, Ludhiana farm (11.8 kg ha^{-1}). Soil was air dried and passed through 100-mesh sieve for soil microbiological studies. Soil physico-chemical properties were determined using 2 mm sieved soil sample. Soil pH was determined in 1:1.25 soil-water suspension with digital pH meter, electrical conductivity, cation exchange capacity, organic matter and available phosphorus were determined using standard AOAC methods. The soil was sandy loam in texture and was reported to be alkaline with pH 8.56, electrical conductivity 0.20 mmhos cm^{-1}, cation exchange capacity 17.39 cmol Kg^{-1} and organic carbon 0.503%.

Immobilisation of bacterial cells in gel beads of carageenan for delivery

The highly efficient bacteria viz. 5*Bacillus*P, 42*Bacillus*P, 5*Bacillus*M, 42 *Bacillus*M and 5*Bacillus*M+42*Bacillus*M were also used in soil for mass multiplication purpose for getting ecological benefits. These bacterial cells were immobilised in defined polymeric gel beads of carageenan for delivery in green house conditions. 10^6 bacterial cells ml^{-1} of 5*Bacillus*P and 42*Bacillus*P, 5*Bacillus*M with 100 µg ml^{-1} Vancomycin, Polymyxin, Erythromycin and 42*Bacillus*M with 100 µg ml^{-1} of Polymyxin, Erythromycin, Ampicillin, Kanamycin and Amoxycillin were inoculated in 100 ml nutrient broth. 5*Bacillus*M and 42*Bacillus*M were co-inoculated in 100 ml nutrient broth supplemented with 100 µg ml^{-1} of Polymyxin and Erythromycin. These inoculated bacteria were incubated for 4 days at 28 ± 2^0C with slight shaking. Cells were separated by centrifugation (10,000 rpm for 10 minutes) and resuspended in solution of K-Carageenan (1.5 % in 0.9 % solution of NaCl). The mixture was extruded dropwise from needle (0.8 mm diameter) in a cooled solution of 3 % KCl. The gel bead formation was carried out for 10-15 minutes. The average diameter of beads was about 2mm (Denkova et al, 2004).

Macrocapsules entrapping simultaneously 5*Bacillus*M and 42*Bacillus*M, showing synergistic interactions were developed to facilitate co-inoculation for enhanced beneficial action.

Influence of gel-entrapped microbial inoculants on plant growth under green house

To study the establishment of PSB, and the resulting effect on crop yield, the soil was air-dried, sieved through 2 mm sieve and filled in polythene-lined pots of 4 Kg capacities. The soil was low in P (11.8 Kg ha^{-1}). Nitrogen and potassium each were applied @ 10 ml per pot as urea (120 mg Kg^{-1}) and KCl (25 mg K Kg^{-1}), respectively whereas the source and rate of phosphorus application were varied. Single super phosphate (SSP) (60 mg Kg^{-1}) and URP (60 mg Kg^{-1} were applied @ 3.4 g per pot and 3 g per pot, respectively for 60 mg Kg^{-1} level while 1.7 g per pot and 1.5 g per pot, respectively for 30 mg Kg^{-1} levels. Wheat var. PBW343 was used as a test crop. Total six seeds along with immobilized bacteria were sown in pots. The moisture status of soil was maintained at field capacity. Plants were irrigated as and when required. The enumeration of PSB was carried out at 20, 35, 50 and 70 days interval (Table 4). The available P of soil was determined at 35 and 70 days (Table 5). The plants were uprooted gently. The data on shoot biomass was recorded at harvest stage. Total nitrogen and phosphorus was determined by standard AOAC methods.

Statistical Analysis

Results were analysed using factorial experiment in CRD and standard deviation was also determined.

RESULTS & DISCUSSION

The antibiotic resistance spectra of different organisms indicated that six PSB were found to show resistance for 3-8 antibiotics viz. 5*Bacillus* (resistance for three), 27M12 *Micrococcus* (resistance for eight), 28 (resistance for four), 29 (resistance for four), 35M11*Micrococcus* (resistance for five), 42*Bacillus* (resistance for five) (Table 1)) at 100µg ml^{-1}.

Comparison of phosphate solubilising efficiency of screened mutants with their parents in culture broth

The P solubilisation values indicated that out of six, two mutants (5*Bacillus*M and 42*Bacillus*M) represented more URP solubilisation (30.0 µg ml^{-1} and 37.2 µg ml^{-1} respectively) than their parents (5*Bacillus*P and 42*Bacillus*P). The combined effect of these two mutants in NBRIP broth along with one ml of antibiotics viz. Polymyxin and Erythromycin for URP dissolution was also more (30.6 µg ml^{-1}) than that of their parents and 5*Bacillus*M but it was lesser than that of 42*Bacillus*M alone (Table 2). It may be attributed to the production of growth promoting substances by 42 *Bacillus*M, which enhanced the efficacy of 5*Bacillus*M for P solubilisation in combination.

Determination of organic acids in liquid media

The selected microbes were also tested for the production of organic acids by paper chromatography. Citric, Gluconic and Oxalic acids were the major organic acids produced as analysed by chromatographic studies (Table 3). Our results are in agreement with the results of a number of scientists (Halder et al, 1990; Gupta et al, 1994; Illmer and Schinner, 1995). Organic acids are well known to play an important role

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TABLE1: Antibiotic resistance spectra of Phosphate solubilising bacteria (at 100 μg ml^{-1})

Isolates	Streptomycin sulphate	Chloramphenicol	Vancomycin	Polymyxin	Erythromycin	Tetracycline	Ampicillin	Kanamycin	Gentamycin	Amoxycillin
2	-	-	+	-	++	-	-	-	-	-
4	-	-	-	+++	-	-	-	-	-	-
5*Bacillus*M	-	-	+++	+	+++	-	-	-	-	-
6	-	-	-	-	+++	-	-	-	++	-
7	-	-	+++	+	++	-	-	-	-	-
9	-	-	-	+	-	-	-	-	-	-
12	-	-	-	++	+++	-	-	-	-	-
15	+	-	-	-	+++	-	-	-	-	-
17	-	-	-	+	-	-	-	-	-	-
22	-	+++	+++	-	-	-	-	-	-	-
27M12 *Micrococcus*	-	++	+++	+++	+++	-	+++	+++	+++	++
28	-	-	-	+++	+++	-	+++	-	-	+++
29	-	-	-	+++	-	-	+++	-	+	+++
30	-	-	-	+++	-	-	-	-	-	
32	-	-	-	+++	+++	-	+++	-	-	+++
34	-	-	+++	-	+++	-	+++	-	-	+++
35M11 *Micrococcus*	-	-	+++	+++	+++	-	+++	-	-	+++
36	-	-	-	+++	-	-	-	-	-	-
37	-	-	-	+++	-	-	-	-	-	-
42*Bacillus*M	-	-	-	+++	+++	-	+++	+++	-	+++
44	-	-	-	++	-	-	-	-	-	-
46	-	-	-	+++	+++	-	-	-	-	+++
47	-	-	-	-	+	-	-	-	-	-

+++ Highly resistant
++ Moderately resistant
+ Resistant

TABLE 2: Tricalcium phosphate and Udaipur rock phosphate solubilisation (µg ml^{-1}) by bacterial isolates in NBRIP broth

Sources of P / Isolates	Tricalcium phosphate	Udaipur rock phosphate
35P11 *Micrococcus*	91.7 ± 1.35	40.2 ± 0.208
27P12 *Micrococcus*	94.9 ± 0.152	45.5 ± 0.458
35M11 *Micrococcus*	88.3 ± 0.472	33.2 ± 1.06
27M12 *Micrococcus*	92.3 ± 2.05	42.7 ± 2.34
5*Bacillus*P	55.7 ± 0.351	23.7 ± 0.624
42*Bacillus*P	79.8 ± 0.264	29.9 ± 0.776
5*Bacillus*M	73.8 ± 0.971	30.0 ± 0.450
42*Bacillus*M	86.2 ± 0.351	37.2 ± 0.709
5*Bacillus*M+42*Bacillus*M	78.7 ± 0.600	30.6 ± 0.585

TABLE 3: Different organic acids produced by Phosphate solubilisers

Isolates	Rf Value	Known organic acid
35P11 *Micrococcus*	0.170	Citric acid
27P12 *Micrococcus*	0.253	Gluconic acid
35M11 *Micrococcus*	0.168	Citric acid
27M12 *Micrococcus*	0.254	Gluconic acid
5*Bacillus*P	0.158	Oxalic acid
42*Bacillus*P	0.253	Gluconic acid
5*Bacillus*M	0.160	Oxalic acid
42*Bacillus*M	0.252	Gluconic acid

TABLE 4: Rhizosphere population of Phosphate solubilising bacteria ($X10^4$ g $soil^{-}$)

Isolates	Sources of P	Levels of P (mg/Kg)	Days			
			20	35	50	70
Uninoculated Control	P	0	3.72	3.76	3.78	3.79
	SSP	30	4.23	4.28	4.27	4.33
		60	4.16	4.22	4.17	4.11
	URP	30	4.10	4.15	4.20	4.16
		60	3.73	3.80	3.79	3.78
5*Bacillus*P	P	0	3.63	3.70	3.56	3.53
	SSP	30	3.73	3.90	3.83	3.83
		60	3.90	4.13	3.33	4.12
	URP	30	3.20	3.70	3.60	3.33
		60	3.20	3.36	3.93	3.23
42*Bacillus*P	P	0	4.36	5.10	5.33	4.66
	SSP	30	5.03	6.43	6.43	5.46
		60	4.66	6.13	6.13	4.76
	URP	30	4.53	5.30	6.13	4.73
		60	4.36	4.33	5.26	4.50
5*Bacillus*M	P	0	3.66	3.66	3.60	3.66
	SSP	30	4.03	4.28	4.16	4.13
		60	4.03	4.17	3.83	3.93
	URP	30	4.03	3.86	3.70	3.93
		60	3.80	3.50	3.23	3.66
42*Bacillus*M	P	0	5.06	5.73	5.93	5.30
	SSP	30	5.60	6.56	6.56	5.73
		60	5.23	6.30	6.26	5.50
	URP	30	5.00	5.63	5.80	5.23
		60	4.90	5.33	5.76	5.03
5*Bacillus*M+42*Bacillus*M	P	0	4.76	5.46	5.63	4.80
	SSP	30	5.26	5.76	5.73	5.53
		60	4.86	5.33	5.13	4.83
	URP	30	4.40	5.33	5.46	4.53
		60	4.06	4.96	5.13	4.06

P= Phosphorus; SSP= Single super phosphate; URP= Udaipur Rock Phosphate

TABLE 5: Available P_2O_5 (Kg ha^{-1}) content of soil

Isolates	Sources of P	Levels of P (mg/Kg)	Available P_2O_5 (Kg/ha)	
			Days	
			35	70
Uninoculated Control	P	0	14.6 ± 0.152	12.0 ± 0.057
	SSP	30	15.3 ± 0.264	12.5 ± 0.100
		60	15.7 ± 0.208	12.7 ± 0.173
	URP	30	15.0 ± 0.264	12.0 ± 0.100
		60	15.3 ± 0.115	12.2 ± 0.057
5*Bacillus*P	P	0	13.9 ± 0.300	12.2 ± 0.200
	SSP	30	14.5 ± 0.251	12.4 ± 0.100
		60	14.7 ± 0.251	12.7 ± 0.251
	URP	30	14.2 ± 0.152	12.1 ± 0.152
		60	14.2 ± 0.251	12.1 ± 0.152
42*Bacillus*P	P	0	17.4 ± 0.208	14.1 ± 0.300
	SSP	30	17.8 ± 0.152	17.0 ± 0.200
		60	18.7 ± 0.208	17.2 ± 0.321
	URP	30	17.3 ± 0.152	15.7 ± 0.378
		60	17.8 ± 0.100	16.3 ± 0.100
5*Bacillus*M	P	0	14.2 ± 0.208	12.3 ± 0.251
	SSP	30	14.7 ± 0.152	12.4 ± 0.152
		60	14.9 ± 0.251	12.6 ± 0.115
	URP	30	14.2 ± 0.251	12.0 ± 0.057
		60	14.4 ± 0.251	12.2 ± 0.152
42*Bacillus*M	P	0	18.2 ± 0.305	15.0 ± 0.208
	SSP	30	18.7 ± 0.152	18.2 ± 0.251
		60	19.2 ± 0.152	18.9 ± 0.152
	URP	30	18.2 ± 0.251	17.1 ± 0.152
		60	18.4 ± 0.251	17.8 ± 0.200
5*Bacillus*M+42*Bacillus*M	P	0	17.8 ± 0.152	14.4 ± 0.404
	SSP	30	17.9 ± 0.173	15.7 ± 0.251
		60	18.3 ± 0.115	16.2 ± 0.305
	URP	30	17.5 ± 0.251	15.4 ± 0.100
		60	17.7 ± 0.208	15.9 ± 0.264

P= Phosphorus; SSP= Single super phosphate; URP= Udaipur Rock Phosphate

At 35 days of sowing	CD (5%)	At 70 days of sowing	CD (5%)
AB	NS	AB	0.205
AC	0.248	AC	0.252
BC	0.143	BC	0.145
ABC	NS	ABC	NS

A $\rightarrow$Treatments, B $\rightarrow$ Sources, C $\rightarrow$ Rates

Enumeration of PSM

Seed inoculation with immobilised PSB viz. 5*Bacillus*P and 5*Bacillus*M represented a negative effect on rhizosphere population as compared to uninoculated control while inoculation with 42*Bacillus*P and 42*Bacillus*M showed a positive effect on rhizosphere population as compared to uninoculated control. 5*Bacillus*M+42*Bacillus*M inoculated treatments also represented increased population counts than uninoculated control but it was lesser than that of 42*Bacillus*M and more than 5*Bacillus*M inoculated treatments representing that 42*Bacillus*M produced some kind of growth promoting substance for 5*Bacillus* and the other native microflora for enhancement of their multiplication resulting in increased population counts in soil. The productiveness of the rhizosphere for PSB may be attributed to the favourable influence exerted by root exudates. This observation is in corrobation with that of some workers (Vancura and Harizlikova, 1972; Chatli et al, 2007) who also reported the positive effect of root exudates viz. amino acids, carbohydrates, organic acids and growth promoting substances on rhizospheric microflora. The negative effect of 5*Bacillus*P and 5*Bacillus*M on microbial population than control may be attributed to production of inhibitory substances, which suppressed the growth of native microflora. However in all these inoculated treatments, the population count increased at 35 days interval while decreased at 50 days interval. The population was maximum in SSP30 mg Kg^{-1} treatment with all the PSB and the number varied between 3.73-5.60 X 10^4 g^{-1} dry soil at 20 days, 3.90-6.56 X 10^4 g^{-1} dry soil at 35 days, 3.83-6.56 X10^4 g^{-1} dry soil at 50 days and 3.83-5.73 X 10^4 g^{-1} dry soil at 70 days. The stimulatory effect of SSP30 mg Kg^{-1} level on bacterial population may be directly due to the increased supply of available P and indirectly through changing the growth rate and metabolic activities of crop plants resulting in more exudates and thereby creating a favourable habitat for the growth and development of these microorganisms (Table 4). The incidence of an increased number of PSB in the rhizosphere of crop plants have been reported (Bopaiah, 1985; Craven and Hayaseka, 1982).

TABLE 6: Shoot Dry Weight (g plant^{-1}) at 70 days of sowing

Isolates	Sources of P (mg/Kg)	Levels of P	Shoot dry weight (g)
Uninoculated Control	P	0	1.06 ± 0.04
	SSP	30	1.60 ± 0.02
		60	1.24 ± 0.14
	URP	30	1.27 ± 0.16
		60	1.17 ± 0.06
5*Bacillus*P	P	0	0.95 ± 0.03
	SSP	30	1.40 ± 0.07
		60	1.08 ± 0.07
	URP	30	1.11 ± 0.09
		60	1.08 ± 0.04
42*Bacillus*P	P	0	1.55 ± 0.04
	SSP	30	1.89 ± 0.07
		60	1.68 ± 0.06
	URP	30	1.68 ± 0.02
		60	1.58 ± 0.03
5*Bacillus*M	P	0	1.01 ± 0.01
	SSP	30	1.57 ± 0.03
		60	1.23 ± 0.14
	URP	30	1.24 ± 0.13
		60	1.14 ± 0.06
42*Bacillus*M	P	0	1.84 ± 0.04
	SSP	30	1.99 ± 0.01
		60	1.94 ± 0.02
	URP	30	1.93 ± 0.02
		60	1.90 ± 0.01
5*Bacillus*M+42*Bacillus* M	P	0	1.55 ± 0.02
	SSP	30	1.64 ± 0.00
		60	1.60 ± 0.02
	URP	30	1.61 ± 0.01
		60	1.59 ± 0.01

P= Phosphorus; SSP= Single super phosphate; URP= Udaipur Rock Phosphate

	CD (5%)
AB	0.610
AC	0.748
BC	0.431
ABC	0.105

Available P content of soil

The available P_2O_5 content of the soil was increased due to 42*Bacillus*P, 42*Bacillus*M and combination of 5*Bacillus*M+42*Bacillus*M inoculation (18.7 Kg ha^{-1}, 19.2 Kg ha^{-1} and 18.3 Kg ha^{-1} respectively) as compared to uninoculated controls after supplementing 60 mg Kg^{-1} SSP at 35 days of sowing, which may be due to solubilisation of insoluble phosphates. With the increase in dose of SSP available P content of soil was also found to increase. The addition of URP at 30 mg Kg^{-1} and 60 mg Kg^{-1} levels resulted in decrease in available P content than that of SSP supplementation. The available soil P was decreased at 70 days of sowing (Table 5). This may be attributed to greater uptake of this nutrient by the plant and the chemical fixation of soluble P in soil (Kanwar and Grewal, 1974; Khalafallah et al, 1982).

Dry weight of shoot, Total N and Phosphorus content

The 42*Bacillus*M increased shoot biomass (1.99 g Plant^{-1}), N uptake (3980.0 mg Plant^{-1}) and P uptake (716.4 mg Plant^{-1}) followed by the synergistic effect of 5*Bacillus*M+42*Bacillus*M at 70 days of sowing after supplementing 30 mg Kg^{-1}SSP. Further higher dose of SSP resulted in decrease in all these parameters. The same was true with the application of URP. Sapatnekar *et al*, 1994 also reported that wheat responded significantly to the graded levels of SSP along with application of P solubilisers. P uptake and P content was augmented due to the production of growth promoting substances by PSM in wheat and other crops leading to increase in plant growth (Gibaly, 1977; Sattar and Gaur, 1997). The isolates 5*Bacillus*P and 5*Bacillus*M showed percentage decrease in all these parameters than those of uninoculated control (Tables 6, 7, 8).

PSB can increase plant uptake of phosphate and alleviate P stress in P deficient soil leading to increased P concentration. Thus, inoculation of PSB along with lower dose of SSP reduces the phosphate fixation by soil fractions and hence enhances the growth rate of plant.

TABLE 7: Concentration of N (%) plant^{-1} and N uptake (mg plant^{-1}) at 70 days of sowing

Isolates	Sources of P	Levels of P (mg/Kg)	Concentration of N (%)	N uptake (mg plant^{-1})
Uninoculated Control	P	0	1.75 ± 0.036	1855.0
	SSP	30	1.95 ± 0.030	3120.0
		60	1.91 ± 0.017	2368.4
	URP	30	1.85 ± 0.015	2349.5
		60	1.88 ± 0.010	2199.6
5*Bacillus*P	P	0	1.71 ± 0.017	1624.5
	SSP	30	1.89 ± 0.010	2646.0
		60	1.86 ± 0.010	2008.8
	URP	30	1.84 ± 0.011	2042.4
		60	1.82 ± 0.020	1965.6
42*Bacillus*P	P	0	1.80 ± 0.005	2790.0
	SSP	30	1.95 ± 0.020	3685.5
		60	1.83 ± 0.010	3074.4
	URP	30	1.81 ± 0.005	3040.8
		60	1.80 ± 0.005	2844.0
5*Bacillus*M	P	0	1.74 ± 0.015	1757.4
	SSP	30	1.93 ± 0.015	3030.1
		60	1.89 ± 0.010	2324.7
	URP	30	1.86 ± 0.015	2306.4
		60	1.84 ± 0.005	2097.6
42*Bacillus*M	P	0	1.85 ± 0.011	3404.0
	SSP	30	2.00 ± 0.010	3980.0
		60	1.87 ± 0.015	3627.8
	URP	30	1.85 ± 0.010	3570.5
		60	1.83 ± 0.005	3477.0
5*Bacillus*M+42*Bacillus*M	P	0	1.82 ± 0.005	2821.0
	SSP	30	1.98 ± 0.005	3247.2
		60	1.85 ± 0.025	2960.0
	URP	30	1.85 ± 0.025	2978.5
		60	1.83 ± 0.010	2909.7

P= Phosphorus; SSP= Single super phosphate; URP= Udaipur Rock Phosphate

	CD (5%)
AB	0.148
AC	0.182
BC	0.105
ABC	0.257

TABLE 8: Concentration of P (%) plant^{-1} and P uptake (mg plant^{-1}) at 70 days of sowing

Isolates	Sources of P	Levels of P (mg/Kg)	Total P (%)	P uptake (mg plant^{-1})
Uninoculated Control	P	0	0.20 ± 0.002	212.0
	SSP	30	0.24 ± 0.000	384.0
		60	0.21 ± 0.002	260.4
	URP	30	0.23 ± 0.005	292.1
		60	0.22 ± 0.009	257.4
5*Bacillus*P	P	0	0.19 ± 0.007	180.5
	SSP	30	0.21 ± 0.007	294.0
		60	0.20 ± 0.008	216.0
	URP	30	0.21 ± 0.010	233.1
		60	0.20 ± 0.012	216.0
42*Bacillus*P	P	0	0.26 ± 0.014	403.0
	SSP	30	0.29 ± 0.007	548.1
		60	0.28 ± 0.005	470.4
	URP	30	0.28 ± 0.002	470.4
		60	0.27 ± 0.005	426.6
5*Bacillus*M	P	0	0.20 ± 0.005	202.0
	SSP	30	0.21 ± 0.011	329.7
		60	0.22 ± 0.005	270.6
	URP	30	0.21 ± 0.007	260.4
		60	0.20 ± 0.002	228.0
42*Bacillus*M	P	0	0.32 ± 0.002	588.8
	SSP	30	0.36 ± 0.012	716.4
		60	0.33 ± 0.004	640.2
	URP	30	0.34 ± 0.010	656.2
		60	0.33 ± 0.002	627.0
5*Bacillus*M+42*Bacillus*M	P	0	0.31 ± 0.005	480.5

	SSP	30	0.35 ± 0.007	574.0
		60	0.32 ± 0.005	512.0
	URP	30	0.34 ± 0.004	547.4
		60	0.33 ± 0.004	524.7

P= Phosphorus; SSP= Single super phosphate; URP= Udaipur Rock Phosphate

	CD (5%)
AB	NS
AC	NS
BC	0.497
ABC	NS

TABLE 9: Correlation matrix of Total N, Total P and dry weight of shoot

	Total N	Total P
Total P	0.384	
Dry weight of shoot	0.543	0.886

Critical value of r at 5% = 0.329

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