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Research Article

PERFORMANCES OF ISOLATED PHOSPHATE SOLUBILIZING BACTERIA ON SOIL MICRO FLORA POPULATION IN GROUNDNUT (*Arachis hypogaea* L.) UNDER VARIED EDAPHIC CONDITIONS

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Abstract- Present study was designed to compare the effect of inoculation with freshly isolated phosphate solubilizing bacterial (PSB), to ones, used as PSB biofertilizer on microbial population *viz*., total bacteria, *Rhizobium* and PSB under *in-vivo* experiments with groundnut (*Arachis hypogaea* L.), under new alluvial and red and lateritic soils. Total bacteria count did not vary significantly due to treatment effects over un-inoculated control pots under both red and laterite and new alluvial soils. Overall, bacterial population was higher in new alluvial soil as compared to red and laterite soils. *Rhizobia* population also exhibited similar trends. However, for both the soils, PSB populations varied significantly, over un-inoculated control at later stages of crop growth.

Keywords- *PSB inoculation, Total Bacteria count, Rhizobium population, PSB population, Pot culture, Groundnut, Red and Laterite soil, New alluvial soil*

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Introduction

Phosphate containing fertilizers that are further added to soil often go through with fixation due to various reactions within the rhizoshere [1]. Huge quantity of phosphatic fertilizer is necessary to be applied in soil every time, in order to sustain crop production [2]. Synthetic fertilizer engages greater cost of production, as well as influence microbial populace within soil harmfully [3]. As an alternative, P-solubilizing micro-organisms can play an important role to circumvent phosphorus deficiencies in more eco-friendly and sustainable manner [4]. It was recommended that phosphate accumulated in agricultural soils are enough to maintain highest yields of crops worldwide for approximately next hundreds of years [5]. Thus, PSB can successfully alter accumulated phosphates into soluble forms for plants [6]. Experiment was conducted involving fresh PSB isolates, obtained initially from eastern India, was screened and evaluated on growth promotion of groundnut, in comparison to existing PSB biofertilizers. The supplementation of elite PSB isolates augmented growth and P-uptake irrespective of edaphic conditions for groundnut [7]. In the second part of this experiment, effect of soil inoculation using fresh or existing PSB test isolates on soil microbial population, *viz*., total bacteria, *Rhizobium* and PSB was evaluated for both new alluvial and red and laterite soil conditions to understand whether such microbial inoculation have impact on soil microbial counts for mentioned bacterial populations.

Materials and Methods

Selection of PSB isolates for the pot culture experiment

Based on P-solubilization assays (experiments on clear halo and quantitative assay of solubilization capacities), top three (03) fresh PSB isolates and one efficient PSB isolate collected from Survey Selection and Mass Production (Nodule Research Centre), Bidhan Chandra Krishi Viswavidyalaya (SAU), West Bengal *viz*., JCA4, JGA5, JMA4 and PSBT3 respectively were selected further for their evaluation under pot culture experiments [Fig-1 and 2].

The performances of the isolates were judged under new alluvial and red and laterite soils.

Fig-1 Selection of Phosphate solubilizing bacteria

Fig-2 P-Solubilizing assay

Preparation of inoculums

PSB isolates for groundnut plants were used for pot experiments. The bacteria were grown in 250mL conical flasks containing 40mL PKV broth for PSB in a shaking incubator for 7 days [Fig-1].

Table-1 *Physico-chemical properties of soils used in pot culture experiment*

A. Physical properties			
Particulars	Values		Method followed
	Red and laterite soil	New-alluvial soil	
Sand (%)	56.7	47.8	International Pipette method [8]
Silt (%)	32.4	31.2	
Clay (%)	9.5	20.8	
B. Chemical properties			
Organic Carbon (%)	0.45	0.58	Walky and Black method [9]
Available N (kg/ha)	37	213.75	Modified Kjeldahl Method [9]
Available phosphorus (kg/ha)	12.3	15.42	Bray Method [10]; Olsen Method [9]
Available potassium (kg/ha)	142.65	167.28	Flame Photometer method [9]
pH	5.2	7.1	Glass electrode Beckman pH meter [9]

Table-2 *Initial microbial population of the experimental soil*

Pot Culture Experiment (2014)		
New Alluvial Soil		Red Soil
Microorganism	Population g^{-1} dry soil	Population g^{-1} dry soil
Total Bacteria	20.1×10^6	11.4×10^6
PSB	3.3×10^2	3.5×10^2
Rhizobium	40×10^4	32.5×10^4

Table-3 Effect of PSB isolates on rhizosphere bacterial population

Treatments	Bacteria population (1×10^5) g^{-1} dry soil							
	Red soil				Alluvial soil			
	Initial	45 DAE	75 DAE	At harvest	Initial	45 DAE	75 DAE	At harvest
JGA4	62	80.5	109	135	62	80.5	109	135
JCA5	59	91	114.5	122.5	59	91	114.5	122.5
JMA4	65	85.5	124	139.5	65	85.5	124	139.5
PSB T3	71.5	82	118.5	131	71.5	82	118.5	131
Control	60	71.5	92	110	60	71.5	92	110
SEm ±	0.536	0.559	0.288	0.379	0.536	0.559	0.288	0.379
CD at 5%	NS	NS	NS	NS	NS	NS	NS	NS

Table-4 Effect of PSB isolates on Rhizobium population

Treatments	*Rhizobium* population (1×10^4) g^{-1} dry soil							
	Red soil				Alluvial soil			
	Initial	45 DAE	75 DAE	At harvest	Initial	45 DAE	75 DAE	At harvest
JGA4	37	47	47	40	53	56	68	81.5
JCA5	32	48.5	51.5	52.5	41.5	60	77.5	93
JMA4	44	39	39	40.5	47	55	69.5	102
PSB T3	32.5	37.5	46	50	34	40.5	59	76
Control	35	34.5	42.5	48	40	46	65	88.5
SEm ±	0.412	0.504	0.606	0.40	0.014	0.504	0.606	0.40
CD at 5%	NS	NS	NS	NS	NS	NS	NS	NS

Table-5 Effect of PSB isolates on PSB population

Treatments	PSB population (1×10^3) g^{-1} dry soil							
	Red soil				Alluvial soil			
	Initial	45 DAE	75 DAE	At harvest	Initial	45 DAE	75 DAE	At harvest
JGA4	0.49	3.2	3.2	3.8	0.39	3.2	3.2	3.3
JCA5	0.33	3.5	4.2	4.3	0.39	3.5	3.7	3.4
JMA4	0.43	3.7	3.8	3.8	0.39	3.3	2.4	3.1
PSB T3	0.35	3.9	3.5	3.7	0.37	3.2	2.8	3.1
Control	0.35	0.4	0.95	0.65	0.38	0.40	0.43	0.51
SEm ±	0.003	0.116	0.645	0.139	0.004	0.255	0.825	0.211
CD at 5%	NS	0.326	1.927	0.415	NS	0.93	3.010	0.77

The cultures were shaken only for 8 hrs each day at 28°-30°C. One milliliter (containing 10^8 cells) of the bacterial culture at their logarithmic stage of growth was inoculated to surface sterilized seeds as per the treatments and dried under shade for 30 minutes prior to sowing. 1% carboxymethyl cellulose (CMC) was added to culture media prior to seed inoculation as adhesive material.

Preparation of Seeds and Soil

Groundnut seeds were surface-sterilized and used for pot experiment. Soils were collected from experimental plots of the regional research station and university seed farms situated under red and laterite zone and new alluvial zones and dried and sieved (2 mm mesh) at room temperature and mixed thoroughly with cow-dung manure in 2:1 ratio for red soil and 3:1 ratio for new alluvial soils and autoclaved twice at interval of 48 hours for complete sterilization [Fig-3 and 4]. The physico-chemical properties of the soils used under pot culture experiment are given in [Table-1].

Pot culture experiment

Surface sterilized seeds were soaked in cultures of PSB isolates for 30 minutes, air dried under shade and were sown in pots ($18 \times 19 cm^2$) containing soils from both new alluvial and red and laterite zones of West Bengal in three replications for each treatment. Pots were placed in green house with temperature range (max/min 27.3/13.7°C) and humidity 85% (8am) to 53% (2pm). Pots were re-inoculated by drenching with specific PSB cultures as per treatments, twice at thirty (30) and fifty (50) days respectively after sowing (DAS).

Fig-3 Preparation of pots with sterile new alluvial soil

Fig-4 Preparation of pots with sterile red and laterite soil

Soil microbial population analysis

Microbial population in terms of total bacteria, *Rhizobium* and PSB of the initial soil samples for both the new alluvial and red and laterite soils, were enumerated using serial dilution and pour plate methods. Total Bacterial population, Rhizobia and PSB population in both soils were also enumerated at 45 DAE (days after emergence), 75 DAE and at harvest of the crop, to judge the population development under both the soils in pot experiments due to application of PSB inoculums.

The microbial population was counted using growth media: nutrient agar (Peptone 5 g/L; Yeast Extract 2 g/L; Sodium Chloride 5 g/L; Agar powder 15 g/L. pH 7.0) for bacteria, YEMA media (Mannitol 10.0 g/L; Yeast extract 1 g/L; K_2HPO_4 0.5 g/L; $MgSO_4.7H_2O$,0.2 g/L; NaCl, 0.1 g/L; Congo red 0.025 g/L; Agar powder 20.0 g /L.) for *Rhizobium* and PKV media (Glucose,10g/L; Tri-calcium phosphate, 5g/L; Ammonium sulphate, 0.5g/L; Potassium chloride 0.2g/L; Magnesium sulphate, 0.1g/L; Manganese sulphate 0.006 g/L; Iron sulphate, 0.006 g/L; Yeast extract 0.5 g/L; Agar powder 15 g/L) for PSB. The initial microbial population for pot culture experiment is given in [Table-2].

Analysis of variance

The data collected from the pot experiment was subjected to statistical analysis appropriate to the design and treatment variations were tested for significance by 'F' test [11, 12]. The standard error of mean and critical difference at 5% level of significance, Fisher and Yates (1963) table were consulted [13].

Results and Discussion

Effect of inoculation of groundnut seeds with selected PSB isolates on soil microbial population is presented in [Tables-3 to 5]. Bacterial population did not vary significantly due to treatment effects over un-inoculated control under both red and laterite and new alluvial soils under pot experiments. However, overall bacterial population increased gradually with progression in the age of crop. This might be due to the effect of root exudates on rhizophere bacterial population. Overall, bacterial population was higher in new alluvial soil as compared to red and laterite soils, which might be due to variation in physico-chemical characteristics, such as, pH, organic carbon%, moisture content, concentration of nutrient element *etc* for both the soils. These factors are considered as major ones affecting the bacterial count of the rhizosphere. Rhizobia population also exhibited similar trends like that of total bacteria. However, for both the soils, PSB populations varied significantly, over un-inoculated control at later stages of crop growth. Such growth effect might be due to PSB population enhancement in rhizosphere of groundnut, because of addition of PSB inoculum at two intervals *i.e.*, at 30 and 50 DAS of the crop growth stages. Highest PSB population was found with fresh isolate JCA5, which was significantly superior to control (no PSB inoculation) for both 45 and 75 DAE and to even known isolate PSBT3 treatment at harvest under red and laterite soil. However, for new alluvial soil, JCA5 performed statistically at par with all other treatments except control pots. This may be due to JCA5, being native to red and laterite soil and being freshly isolated from the same soil zone performed better over known isolate PSBT3 in that soil, while under new alluvial soil condition both performed statistically at par, though JCA5 indicated better PSB population for all observations.

Conclusion

This study confirmed that the inoculums of PSB can play an important role in augmenting PSB population in soils, which in turn can improve the phosphate solubilization activities and thereby can help in plant growth promotion.

Application of research: It was also evident that native isolates perform better in their native edaphic conditions compared to other zones, specially pertaining to varied soil physico-chemical properties for different zones. These findings allow us a new scope for extensive research in Agricultural Microbiology and Biotechnology.

Research Category: Agronomy

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Study area / Sample Collection: Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, 741252, West Bengal, India

Cultivar / Variety / Breed name: Groundnut (*Arachis hypogaea* L.)

Conflict of Interest: None declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.
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