



Effect of phosphorus solubilising bacteria on physiological and biochemical parameters in groundnut (*Arachis hypogaea*)

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ABSTRACT

The phosphorus use efficiency is low in groundnut (*Arachis hypogaea* L.) due to phosphorus fertilisers in soil rapidly form insoluble complexes with metals like Fe, Al, Ca, and Mg. Phosphate solubilising bacteria (PSB) including *Azotobacter*, *Bacillus*, *Pseudomonas*, and their consortium enhance P availability through organic acid production and phosphatase activity, improving plant uptake and yield. Thus, this study evaluated seed treatments with PSB at recommended (50 kg/ha single superphosphate equivalent) and double P doses to assess physiological and biochemical parameters at 70 days after sowing. The experiment was conducted during rainy (*kharif*) season 2021 and 2022 at Punjab Agricultural University, Ludhiana, Punjab using groundnut variety 'SG99' in a randomised block design. The PSB inoculants significantly boosted chlorophyll a (up to 1.91 mg/g FW with *Azotobacter* double dose), total chlorophyll (2.36 mg/g FW), carotenoids (0.486 mg/g FW), leghaemoglobin (3.56 mg/g nodule FW with *Bacillus* double dose), enzyme activities (nitrite reductase 0.982 $\mu\text{mol NO}_2^-$ released/h/g FW; nitrate reductase 0.369 $\mu\text{mol NO}_2^-$ formed/h/g FW), relative water content (70.70%), and membrane stability (76.93%) compared to controls. Total sugars reached 15.59 mg/g DW with *Bacillus* double dose. Thus, *Bacillus* and *Azotobacter* may be recommended in groundnut for sustainable production and quality.

Keywords: *Bacillus*, Bacteria consortium, PSB, Sustainable production

Phosphorus (P) is a critical macronutrient essential for plant metabolic processes, including respiration, photosynthesis, energy transfer, and the synthesis of chlorophyll, vitamins, glucosides, ferredoxins, and disulphide linkages (Khan *et al.* 2023). However, applied P fertilisers rapidly react with soil constituents and converted into less soluble or insoluble forms, leading to low phosphorus use efficiency (PUE) of only 20–25% during the crop growing season and excessive soil P accumulation (Marschner 2012). Groundnut (*Arachis hypogaea* L.), a self-pollinated autotetraploid legume (2n=40), covers 26.4 mha globally with 37.1 mt output (Faldu *et al.* 2018) is highly sought for its oil content. Therefore, groundnut has elevated P demands to support nodulation, oil synthesis, and overall productivity (Shiyam 2010). Phosphate-solubilising bacteria (PSB) such as strains from *Pseudomonas*, *Bacillus*, *Azotobacter*, *Rhizobium*, and others (e.g. *Beijerinckia*, *Burkholderia*) dominate the rhizosphere, a nutrient-rich zone surrounding roots that fosters microbial activity and plant

growth (Singh *et al.* 2010, Taurian *et al.* 2010, Bhattacharyya and Jha 2012, Gianfreda 2015). In groundnut (peanuts), phosphorus also plays a significant role in supporting root and lateral root formation, which is essential for optimal vegetative growth (Bajya and Sharma 2024). The PSB inoculation has consistently improved crop physiological traits enhancing relative water content, membrane stability, leghaemoglobin, nodulation, photosynthetic pigments, and enzymes like nitrite and nitrate reductases across P doses (Kalayu 2019). While prior studies affirm PSB's role in elevating yield and soil health via plant growth-promoting rhizobacteria (PGPR) activities (Bhattacharyya and Jha 2012), gaps persist in dose-response effects of consortium PSB on groundnut under field conditions, especially physiological and biochemical responses at varying P levels and integrated assessments in Indian agroecosystems remain limited (Sharma *et al.* 2025). Thus, this study aimed to evaluate PSB seed treatments on groundnut and their effect on pigments, relative water content, membrane stability, leghaemoglobin, enzyme activities, sugars, and proteins to check the sustainable P management.

MATERIALS AND METHODS

The experiment was conducted during rainy (*kharif*) season 2021 and 2022 at Punjab Agricultural University

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Table 1 Chemical properties of soil at experimental site

Soil property	Before sowing		After harvesting		Reference
	2021	2022	2021	2022	
pH	6.87	6.51	6.89	6.88	Richards (1954)
EC (dS/m)	0.332	0.338	0.452	0.420	Jackson (1973)
Organic carbon (%)	0.445	0.483	0.412	0.420	Walkley and Black (1934)
Nitrogen (kg/ha)	76.9	79.5	82.6	85.2	Subbiah and Asija (1956)
Phosphorus (kg/ha)	14.1	15.6	16.5	17.1	Olsen <i>et al.</i> (1954)
Potassium (kg/ha)	136	139	140	143	Jackson (1974)

(PAU), Ludhiana (30°40'N, 74°44'E; at an elevation of 247 m amsl), Punjab. The experiment was laid out in random block design in 10 m × 10 m plot, replicated thrice. The 'SG99' variety of groundnut was taken for the study. Treatments included control [recommended dose of single super phosphate (SSP) @50 kg/ha (EP1)], Double dose of SSP @100 kg/ha (EP2), *Azotobacter* @single recommended dose (AP1), *Azotobacter* @double dose (AP2), *Bacillus* @single recommended dose (BP1), *Bacillus* @double dose (BP2), *Pseudomonas* @single recommended dose (CP1), *Pseudomonas* @double dose (CP2), Consortium @single recommended dose (DP1), and Consortium @double dose (DP2). Phosphorous solubilising bacteria (PSB) were procured from the Department of Microbiology, PAU, Ludhiana. The status of soil properties before and after harvest with recommended method are given in Table 1.

Preparation of solution of culture and seed treatment: The inoculations were prepared about 1 h before commencement of sowing using microbial strains (*Azotobacter*, *Bacillus* and *Pseudomonas*). A solution of jaggery (10%) was prepared and 1 L of solution was used to treat the 100 kg of groundnut seed. A 200 g of culture of microbial inoculum containing 10⁹–10¹⁰ cells/g carriers were mixed with 10% cold solution of jaggery to make slurry. The seeds were mixed with slurry of inoculant strains on hard floor on polythene sheet under shade conditions.

Physiological parameters analysis

SPAD value: The chlorophyll content was measured using chlorophyll meter (SPAD502 plus) in 4th leaf from the top (Fully expanded leaflet). The SPAD value measured at flowering and podding stage in 10 plants/plot and was expressed in term of SPAD units.

Photosynthetic pigments: Hiscox and Israelstam (1979) method was used to estimate the photosynthetic pigments. A 0.1 g leaf sample dipped in 5 mL of DMSO in a vial and stored overnight. Then the vial was kept in boiling water bath at 65°C for 1 h and the absorbance was recorded in UV 2,600 spectrophotometer (Techcomp) at 645 nm, 663 nm and 480 nm. The amount of total chlorophyll, chlorophyll a, and b was estimated by Arnons equations and carotenoids was calculated using Kirk and Allen (1965) equation.

$$\text{Chl a (mg/g FW)} = 12.7 \times A_{663} - 2.69 \times \frac{\text{volume}}{1000 \times \text{weight (g)}}$$

$$\text{Chl b (mg/g FW)} = 22.9 \times A_{645} - 4.68 \times A_{663} \times \frac{\text{volume}}{1000 \times \text{weight (g)}}$$

$$\text{Total Chl (mg/g FW)} = 20.2 \times A_{645} + 8.02 \times A_{663} \times \frac{\text{volume}}{1000 \times \text{weight (g)}}$$

$$\text{Carotenoids (mg/g FW)} = \frac{1000 \times A_{480} - 1.29 \times \text{Chl a} - 53.78 \times \text{Chl b}}{220} \times \frac{\text{volume}}{1000 \times \text{weight (g)}}$$

Where A, Absorbance.

Relative water content (%): Weatherly (1950) method was used to estimate relative water content (RWC). From 3rd and 4th fully expanded uniform 5 discs, leaf sample was taken in each replication and weight as fresh weight (FW) of leaves. The leaf discs were put in distilled water for 4 h at 25°C in culture tubes and recorded the turgid weight (TW) after 4 h dip. Later, the leaf discs were kept for drying at 60°C for 48 h and recorded the dry weight (DW).

$$\text{Relative water content (RWC\%)} = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100$$

Membrane stability index: Premachandra *et al.* (1990) method was used to determine the leaf membrane stability index (MSI). The 0.1 g of 3rd or 4th leaves strips were placed in 10 mL of distilled water in test tubes and incubated at room temperature for 4 h. Using the microprocessor conductivity meter, electrical conductivity (EC) was measured. Then, in boiling water bath, the test tubes were placed for 1 h and measured conductivity (C2) after cooling. Membrane injury (MI) and membrane stability index were calculated using the following formulae:

$$\text{Membrane stability (MSI\%)} = [1 - \text{C1} / \text{C2}] \times 100$$

$$\text{Membrane injury (MI\%)} = 100 - \text{MSI}$$

Biochemical parameters:

Leghaemoglobin content: Wilson and Reisenauer (1963) method was used to determine the leghaemoglobin content. Fresh nodule (0.5 g), collected at 70 days after sowing

(DAS), was crushed in a chilled mortar and pestle with 3 mL Drabkin's solution. Drabkin's solution was prepared by dissolving 198 mg of potassium ferricyanide, 1 g of sodium bicarbonate and 52 mg of potassium cyanide in 1 L of distilled water. The volume of crushed sample was made up to 7 mL and centrifuged at 5,000 rpm for 25 min. After, the clear supernatant was collected and made the 10 mL final volume with Drabkin's reagent. The absorbance was measured at 540 nm using Drabkin's solution as a reference. The leghaemoglobin content was calculated using calibration curve of known concentration of cyano-methaemoglobin and expressed as mg/g FW.

Nitrite reductase activity: Verner and Ferari (1971) protocol was used to calculate nitrite reductase activity (NIR). About 200 mg tissue was suspended in 2 mL of chloramphenicol, NaNO_2 , and 0.1 M sodium phosphate buffer (pH 4.5). Immediately, 0.1 mL aliquot was used to calculate the initial NO_2^- concentration in the medium. Again, 0.1 mL aliquot was taken for NO_2^- determination after 40 min. The amount of NO_2^- taken up by the tissue was determined by the difference between initial and final concentration of NO_2^- in the medium. Then added the dimethylsulfoxide (50% v/v) and the vials were put on a hot plate until the medium boiling started. Under boiling conditions, the NO_2^- of tissue, rapidly leaked into the medium. For NO_2^- determination, 0.2 mL aliquot was taken after cooling. The difference between this NO_2^- concentration and the initial concentration measures the amount of NO_2^- reduced. Calorimetrically, the nitrite concentration was determined by adding 1 mL of each 0.02% NEDD and 1% sulphanilamide in 3N HCl to 0.1 mL aliquot. Later, it was centrifuged at 2,000 rpm for 10 min and absorbance

was recorded at 540 nm. The standard curve was prepared using 0–10 μg of NaNO_2 . Nitrite reductase activity was expressed as $\mu\text{mol NO}_2^-$ released/h/g FW.

Nitrate reductase activity: Jaworski (1971) protocol was used to determine the nitrate reductase activity (NR). Fresh tissue (200 mg) was suspended in 0.05 mL of n-propanol, 0.25 g KNO_3 and 5 mL of 0.1 M phosphate buffer (pH 7.5). To make the incubation medium free from atmospheric O_2 , nitrogen gas was passed. After sealing of vials, they were incubated in a metabolic shaker in the dark at 30°C for 90 min. The nitrate released in the medium was measured by treating 1 mL of aliquot with 0.02% NEDD and 1 mL of 1% sulphanilamide and made the volume 10 mL with distilled water. The absorbance was measured at 540 nm after 20 min in spectrophotometer. The standard curve was prepared using 0–10 μg of KNO_2 . Nitrate reductase activity was expressed as $\mu\text{mol NO}_2^-$ formed/h/g FW.

Total sugar estimation: Total sugars were estimated using the protocol described by Yemm and Willis (1954). The extent of accuracy in the quantitative estimation has a direct bearing on the extraction procedure applied to the original raw plant material. In the present study, 1 g of the dried material was taken in thick-walled Pyrex test tubes. About 25 mL of distilled water was added to each tube. The content was heated in boiling water and filtered through Whatman No. 1 filter paper. The material which settled in the tube was extracted again with distilled water (about 20 mL) for 10 min and superannuated. This process of extraction was protested 10 times after which no sugar components could be detected in the residue, as tested with anthrone reagent (Yemm and Willis 1954). All these filtrations were done in a standard volumetric flask.

Table 2 Effect of seed treatment and P on photosynthetic pigments in SG99 variety at 70 DAS

Treatments	Chlorophyll a (mg/g FW)			Chlorophyll b (mg/g FW)			Total chlorophyll (mg/g FW)			Carotenoids (mg/g FW)		
	2021	2022	Pooled mean	2021	2022	Pooled mean	2021	2022	Pooled mean	2021	2022	Pooled mean
EP1	1.67	1.03	1.35	0.395	0.350	0.372	2.07	1.38	1.72	0.378	0.363	0.371
EP2	1.49	1.09	1.29	0.334	0.369	0.352	1.82	1.46	1.64	0.382	0.370	0.376
AP1	1.69	1.42	1.55	0.363	0.508	0.436	2.05	1.93	1.99	0.477	0.437	0.457
AP2	1.68	2.14	1.91	0.335	0.569	0.452	2.02	2.71	2.36	0.483	0.443	0.463
BP1	1.90	1.33	1.61	0.408	0.473	0.440	2.31	1.80	2.05	0.493	0.385	0.439
BP2	1.66	1.37	1.52	0.407	0.499	0.453	2.07	1.87	1.97	0.451	0.475	0.463
CP1	1.63	1.16	1.40	0.389	0.434	0.411	2.02	1.59	1.81	0.407	0.478	0.443
CP2	1.76	1.26	1.51	0.405	0.454	0.429	2.17	1.71	1.94	0.460	0.490	0.475
DP1	1.79	1.11	1.45	0.474	0.399	0.436	2.26	1.51	1.89	0.478	0.452	0.465
DP2	1.77	1.14	1.45	0.498	0.410	0.454	2.27	1.55	1.90	0.490	0.482	0.486
Average	1.70	1.30	1.50	0.401	0.447	0.424	2.10	1.75	1.93	0.458	0.446	0.452
CD ($p=0.05$)	0.085	0.0457	Y=0.023, T=0.053, Y $\times$ T=0.074	0.0166	0.015	Y=0.005, T=0.012, Y $\times$ T=0.017	0.058	0.078	Y=0.017, T=0.039, Y $\times$ T=0.055	0.016	0.014	Y=0.007, T=0.015, Y $\times$ T=0.021

T, Treatment; Y, Year; NS, Non-significant; DAS, Days after sowing. Treatment details are given under Materials and Methods.

RESULTS AND DISCUSSION

Seed treatments with phosphate-solubilising bacteria (PSB) significantly enhanced chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents in groundnut leaves at 70 DAS, with variations across years and P doses (Table 2). Pooled means showed maximum chlorophyll a (1.91 mg/g FW) under *Azotobacter* at double P dose (AP2), compared to the control at recommended P (EP1; 1.35 mg/g FW). Chlorophyll b peaked at 0.454 mg/g FW with consortium at double P (DP2) while total chlorophyll reached 2.36 mg/g FW under AP2 vs. 1.64 mg/g FW in control double P (EP2); carotenoids were highest at 0.486 mg/g FW with DP2 (Table 2). These improvements may be due to PSB mediated phosphate solubilisation which boosted P availability for chlorophyll biosynthesis and photosynthesis (Ali *et al.* 2025). Similarly, chlorophyll content was reported with multi-microbial inoculants like *Glomus mosseae* + *Pseudomonas fluorescens* in groundnut. Zaid and Khan (2006) reported that triple inoculations enhanced foliage chlorophyll (24%) in green gram.

The PSB inoculants improved RWC (up to 70.70%), membrane stability (76.93%), and SPAD values (50.83) with AP2, outperforming P controls (RWC 61.13%, stability 66.15%, SPAD 42.64% at EP1). Membrane injury was lowest (23.08%) under AP2, indicating better stress tolerance (Table 3). These results might be due to the enhanced P nutrition from PSB likely strengthening cell membranes and water retention via osmolyte accumulation, consistent with prior PGPR effects reducing injury in legumes (Iftikhar *et al.* 2023).

Nitrite reductase activity peaked at 0.982 $\mu\text{mol NO}_2^-$ released/h/g FW with *Bacillus* double P (BP2) compared to 0.820 in EP1 control. Nitrate reductase was highest (0.369 $\mu\text{mol NO}_2^-$ formed/h/g FW) under *Pseudomonas* double P (CP2), doubling control levels (Table 4). Also, Tariq *et al.* (2014) found increase of nitrite reductase activity by 10.12% in chickpea with PSB + P treatment. These results might be due to supplementation of P, facilitating N metabolism via enzyme synthesis (Table 4).

Leghaemoglobin content in nodules was markedly higher (3.56 mg/g FW at BP2) than controls (2.24 mg/g FW at EP1), with CP2 at 3.43 mg/g FW (Table 4). Similarly, Chaouki *et al.* (2026) found 53% increases in leghaemoglobin in chickpea under integrated P + PSB. This reflects PSB promotion of nodulation and N_2 fixation via better P supply to rhizobia which supports symbiotic efficiency.

Total sugars reached 15.59 mg/g DW under BP2 followed by CP1 (15.09 mg/g DW), than 10.77 mg/g DW in DP1; total proteins peaked at 15.16 mg/g FW (EP2 control) but remained elevated (13.83 mg/g FW, AP2) under PSB (Table 5). Ye *et al.* (2025) demonstrated that optimal fertilisation enhances yield and quality through coordinated regulation of photosynthesis and carbohydrate metabolism. Also, Song *et al.* (2022) showed that enhanced carbohydrates indicate improved photosynthesis and translocation, while proteins link to N assimilation; PSB thus optimise source-sink dynamics in P-limited groundnut.

Total protein content in groundnut leaves at 70 DAS varied significantly across treatments and years, ranging from 10.64 mg/g FW (DP1) to 14.96 mg/g FW (EP2) in *kharif* 2021, and 10.19 mg/g FW (DP2) to 15.36 mg/g FW

Table 3 Effect of seed treatment and P on relative water content, membrane stability, membrane injury and SPAD values in SG99 at 70 DAS

Treatment	Relative water content (%)			Membrane stability (%)			Membrane injury (%)			SPAD-values		
	2021	2022	Pooled mean	2021	2022	Pooled mean	2021	2022	Pooled mean	2021	2022	Pooled mean
EP1	67.48	54.78	61.13	66.75	65.55	66.15	33.25	34.45	33.85	42.11	43.17	42.64
EP2	63.02	59.45	61.24	67.55	66.18	66.86	32.45	33.82	33.14	43.62	44.63	44.12
AP1	64.72	68.00	66.36	77.71	75.01	76.36	22.29	24.99	23.64	50.73	50.77	50.75
AP2	71.30	70.10	70.70	78.33	75.52	76.93	21.67	24.48	23.08	50.87	50.8	50.83
BP1	66.9	65.85	66.37	75.79	72.65	74.22	24.21	27.35	25.78	47.74	47.77	47.76
BP2	63.02	67.43	65.23	77.11	74.71	75.91	22.89	25.29	24.09	49.63	49.73	49.68
CP1	61.89	65.17	63.53	72.04	72.21	72.13	27.96	27.79	27.88	47.23	47.33	47.28
CP2	64.81	65.5	65.16	74.95	72.31	73.63	25.05	27.69	26.37	46.52	47.57	47.05
DP1	66.31	59.45	62.88	68.77	70.03	69.4	31.23	29.97	30.6	44.67	44.63	44.65
DP2	71.44	62.88	67.16	70.78	71.16	70.97	29.22	28.84	29.03	46.84	46.87	46.85
Average	66.09	63.86	64.98	72.98	71.53	72.26	27.02	28.47	27.75	47.00	47.33	47.16
CD ($p=0.05$)	3.160	2.363	Y=0.875, T=1.957, Y $\times$ T=2.767	3.626	3.200	Y=1.042, T=2.329, Y $\times$ T=NS	1.198	1.008	Y=0.332, T=0.743, Y $\times$ T=1.051	2.306	2.281	Y=NS, T=1.473, Y $\times$ T=NS

T, Treatment; Y, Year; NS, Non-significant; DAS, Days after sowing. Treatment details are given under Materials and Methods.

Table 4 Effect of seed treatment and phosphorous on nitrite reductase, nitrate reductase in leaves and leghaemoglobin at 70 DAS

Treatments	Nitrite reductase ($\mu\text{mol No}_2^-$ released/h/g FW)			Nitrate reductase ($\mu\text{mol No}_2^-$ formed/h/g FW)			Leghaemoglobin (mg/g nodule FW)		
	2021	2022	Pooled mean	2021	2022	Pooled mean	2021	2022	Pooled mean
EP1	0.815	0.824	0.820	0.326	0.321	0.324	2.27	2.21	2.24
EP2	0.817	0.834	0.826	0.327	0.329	0.328	2.35	2.45	2.40
AP1	0.932	0.980	0.956	0.352	0.332	0.342	2.46	2.68	2.57
AP2	0.981	0.940	0.961	0.329	0.364	0.347	3.40	3.20	3.30
BP1	0.900	0.945	0.923	0.341	0.339	0.340	2.63	2.43	2.53
BP2	0.988	0.975	0.982	0.353	0.358	0.356	3.54	3.58	3.56
CP1	0.927	0.998	0.963	0.336	0.382	0.359	2.46	2.56	2.51
CP2	0.982	0.971	0.977	0.370	0.367	0.369	3.48	3.38	3.43
DP1	0.845	0.883	0.864	0.358	0.355	0.357	2.75	2.27	2.51
DP2	0.869	0.877	0.873	0.355	0.361	0.358	2.68	2.59	2.64
Average	0.906	0.923	0.914	0.345	0.351	0.348	2.80	2.74	2.77
CD ($p=0.05$)	0.041	0.047	Y=0.007, T=0.015, Y $\times$ T=0.021	0.014	0.011	Y=0.004, T=0.008, Y $\times$ T=0.011	0.87	0.98	Y=0.036, T=0.080 Y $\times$ T= 0.113

T, Treatment; Y, Year; NS, Non-significant; DAS, Days after sowing. Treatment details are given under Materials and Methods.

Table 5 Effect of seed treatment and P on total sugar and protein in leaves at 70 DAS

Treatments	Total sugar (mg/g DW)			Total protein (mg/g FW)		
	2021	2022	Pooled mean	2021	2022	Pooled mean
EP1	15.69	10.47	13.08	13.79	14.16	13.98
EP2	12.45	12.08	12.27	14.96	15.36	15.16
AP1	17.6	10.95	14.28	12.9	13.07	12.98
AP2	17.69	9.51	13.60	13.7	13.96	13.83
BP1	15.10	9.11	12.11	12.17	12.89	12.53
BP2	21.17	10.01	15.59	13.41	13.76	13.58
CP1	18.07	12.12	15.09	11.82	11.47	11.64
CP2	17.51	10.01	13.76	12.57	12.91	12.74
DP1	11.67	9.86	10.77	10.64	10.26	10.45
DP2	14.83	11.5	13.17	11.07	10.19	10.63
Average	16.18	10.56	13.37	12.70	12.80	12.75
CD ($p=0.05$)	0.867	0.440	Y=0.215, T=0.481, Y $\times$ T=0.680	0.609	0.695	Y=0.096, T=0.215, Y $\times$ T=0.305

T, Treatment; Y, Year; NS, Non-significant; DAS, Days after sowing. Treatment details are given under Materials and Methods.

(EP2) in *kharif* 2022 (Table 4). Pooled means indicated the highest protein at 15.16 mg/g FW (EP2), followed by AP2 (13.83 mg/g FW) and BP2 (13.58 mg/g FW) with the minimum at 10.45 mg/g FW (DP1). The PSB treatments sustained high protein levels close to double P controls, likely due to enhanced N assimilation from improved nodulation

and enzyme activities (nitrate/nitrite reductases) (Table 5), channelling amino acid synthesis despite lower direct P input (Bargaz *et al.* 2021). Also, same results were reported that PSB inoculation increased kernel protein by up to 24.25% in groundnut through better nutrient mobilisation (Sharma *et al.* 2025).

Table 6 Effect of seed treatment and phosphorus on phosphorous in kernels at physiological maturity and pod yield in groundnut

Treatments	Phosphorous content (%)						Pod yield (kg/ha)		
	Kernel			Shoot					
	2021	2022	Pooled mean	2021	2022	Pooled mean	2021	2022	Pooled mean
EP1	0.500	0.494	0.497	0.274	0.380	0.327	2,522	2,635	2,579
EP2	0.520	0.550	0.535	0.282	0.350	0.316	2,545	2,677	2,611
AP1	0.505	0.530	0.518	0.310	0.300	0.305	2,956	3,082	3,019
AP2	0.515	0.515	0.515	0.320	0.320	0.320	2,976	3,196	3,086
BP1	0.520	0.570	0.545	0.330	0.290	0.310	2,764	2,880	2,822
BP2	0.550	0.545	0.548	0.340	0.310	0.325	2,898	2,791	2,845
CP1	0.515	0.555	0.535	0.300	0.300	0.300	2,760	2,711	2,736
CP2	0.530	0.515	0.523	0.330	0.320	0.325	2,879	2,786	2,833
DP1	0.560	0.535	0.548	0.340	0.300	0.320	2,880	2,972	2,926
DP2	0.570	0.520	0.545	0.360	0.320	0.340	2,998	2,895	2,947
Average	0.529	0.533	0.531	0.319	0.319	0.319	2,818	2,863	2,840
CD ($p=0.05$)	0.027	0.020	Y=0.027, T=0.012, Y × T=0.002	0.012	0.019	Y=0.040, T=0.025, Y × T=0.034	122.0	146.8	Y=40.69, T=91.01, Y × T=128.7

T, Treatment; Y, Year; NS, Non-significant; DAS, Days after sowing. Treatment details are given under Materials and Methods.

During *kharif* 2021, phosphorous content (%) significantly varied between kernel and shoot from 0.5% (EP1) to 0.570% (DP2) and 0.274 (EP1) to 0.360 (DP2), respectively. While during *kharif* 2022, it varied from 0.494 (EP1) to 0.570% (BP1) and 0.290 (BP1) to 0.38% (EP1), respectively. Pooled mean analysis showed that in kernel, the maximum phosphorous content (%) observed with DP1 and BP2 i.e. 0.548% while minimum recorded with EP1 i.e. 0.497 whereas shoot phosphorous content (%) was higher in DP2 i.e. 0.340 and minimum recorded with CP1 (0.300) (Table 6).

During *kharif* 2021, higher pod yield (kg/ha) achieved with DP2 i.e. 2,998 kg/ha and minimum with EP1 (2,522 kg/ha) whereas during *kharif* 2022, higher pod yield observed with AP2 (3,196 kg/ha) and minimum with EP1 (2,635 kg/ha). Pooled mean analysis showed that pod yield ranged from 2,579 and 3,086 kg/ha and higher pod yield achieved with AP2 i.e. 3,086 kg/ha followed by AP1 (3,019 kg/ha) and DP2 (2,947 kg/ha) (Table 6).

In conclusion, PSB as seed treatment in groundnut significantly enhanced photosynthetic pigments (chlorophyll a: 1.91 mg/g FW; total: 2.36 mg/g FW under *Azotobacter* double dose), RWC (70.70%), membrane stability (76.93%), leghaemoglobin (3.56 mg/g nodule FW), N-assimilation enzymes, sugars (15.59 mg/g DW), and proteins. Thus, *Azotobacter* improves P use efficiency via solubilisation and rhizobial synergy for sustainable groundnut production.

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