

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(8): 1972-1976
www.biochemjournal.com
Received: 07-05-2026
Accepted: 09-06-2026

Sumit Kumar
Department of Soil Science and
Agricultural Chemistry, Chandra
Shekhar Azad University of
Agriculture and Technology,
Kanpur, Uttar Pradesh, India

Sarvesh Kumar
Department of Soil Science and
Agricultural Chemistry, Chandra
Shekhar Azad University of
Agriculture and Technology,
Kanpur, Uttar Pradesh, India

Rohit Kumar Jaiswal
Department of Soil Science and
Agricultural Chemistry, Chandra
Shekhar Azad University of
Agriculture and Technology,
Kanpur, Uttar Pradesh, India

Ajay Kumar Yadav
Department of Soil Science and
Agricultural Chemistry, Sri Karan
Narendra Agriculture University,
Jobner, Rajasthan, India

Tapasya Tiwari
Department of Soil Science and
Agricultural Chemistry, Chandra
Shekhar Azad University of
Agriculture and Technology,
Kanpur, Uttar Pradesh, India

Rupak Kumar
Department of Soil Science and
Agricultural Chemistry, Chandra
Shekhar Azad University of
Agriculture and Technology,
Kanpur, Uttar Pradesh, India

Kapil Kumar Yadav
Department of Soil Science and
Agricultural Chemistry, Chandra
Shekhar Azad University of
Agriculture and Technology,
Kanpur, Uttar Pradesh, India

Umesh Kumar
Department of Soil Science, Sardar
Vallabhbhai Patel University of
Agriculture and Technology
Meerut, Uttar Pradesh, India

Corresponding Author:
Sumit Kumar
Department of Soil Science and
Agricultural Chemistry, Chandra
Shekhar Azad University of
Agriculture and Technology,
Kanpur, Uttar Pradesh, India

Synergetic effect of phosphorus, sulphur and bio-inoculants on soil enzymatic activities and microbial population under chickpea (*Cicer arietinum* L.)

Sumit Kumar, Sarvesh Kumar, Rohit Kumar Jaiswal, Ajay Kumar Yadav, Tapasya Tiwari, Rupak Kumar, Kapil Kumar Yadav and Umesh Kumar

DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i8Sv.9699>

Abstract

A field experiment was conducted during *Rabi* seasons of 2024-25 and 2025-26 at the Student Instructional Farm (SIF), Chandra Shekhar Azad University of Agriculture & Technology, Kanpur, (U.P.). The investigation was undertaken to studies on the effect of phosphorus, sulphur and bio-inoculants on soil properties, growth and yield of Chickpea (*Cicer arietinum* L.) in Central Plain Zone of Uttar Pradesh. The treatments consisted three levels of (P₀: control, P₁: phosphorus @ 30 kg ha⁻¹, P₂: phosphorus @ 60 kg ha⁻¹), three levels of sulphur (S₀: control, S₁: sulphur @ 20 kg ha⁻¹, S₂: sulphur @ 40 kg ha⁻¹) and two levels of bio-inoculants (B₀: control, B₁: inoculation with *Rhizobium* + PSB). The experiment was replicated thrice in a factorial randomized block design (FRBD). The results revealed that application of different treatments affect the alkaline phosphatase activity, soil dehydrogenase and urease enzymes and microbial population of soil after the harvest of chickpea crop were improved significantly due to the integration of phosphorus, sulphur and bio-inoculants. The treatments that included phosphorus, sulphur and *Rhizobium* + PSB showed the greatest improvements in soil health, as evidenced by the increased biological count and soil enzyme activities. Integrated nutrient management involving chemical fertilizers, biofertilizers, and biological inputs has been recommended for maintaining soil fertility and ensuring long-term sustainability.

Keywords: Bio-inoculants, chickpea, phosphorus, soil enzyme activity, total microbial count

1. Introduction

Chickpea (*Cicer arietinum* L.) is a primary leguminous crop cultivated for its edible seeds, belonging to the genus *Cicer*, within the Fabaceae family (Leguminaceae) and the subfamily Papilionaceae. It is predominantly cultivated as a *Rabi* crop in India's regions known for pulse cultivation, Originating from the southeastern region.

Most of the phosphorus present in the soil is unavailable to plants, which is made available through the activities of efficient microorganisms like bacteria, fungi and even cyanobacteria with production of organic acid and increasing Phosphatase enzyme activity. Phosphatic fertilizers which are routinely applied in soil to promote crop yields readily react with calcium and become progressively less available to plants. Only about 15-20 percent of the applied phosphorus is utilized by first crop. Hence, the current trend throughout the world is to explore the possibility of using alternate nutrients sources for increasing the efficiency of chemical fertilizers. Since phosphorus availability from reserve phosphate i.e. rock phosphate in neutral and alkaline soil is very low or negligible. Therefore, the phosphate solubilizing organisms dissolved interlocked phosphate appears to have an important implication in Indian agriculture.

Application of phosphorus and sulphur along with beneficial microorganisms positively influences soil properties by improving microbial biomass, enzyme activities, nutrient cycling, organic carbon dynamics, and nutrient availability. Such integrated approaches also enhance fertilizer use efficiency and reduce nutrient losses.

Bio-inoculants are formulations containing beneficial microorganisms that improve nutrient availability, plant growth, and soil health through biological processes. They are increasingly recognized as an eco-friendly alternative to excessive dependence on chemical fertilizers.

Biofertilizers have been proved as the cheapest source of N and P for better crop yields, particularly in legumes. Indian soils, in general are lacking in effective and specific strains of *Rhizobium* which are responsible for symbiotic nitrogen fixation. Information regarding its effect on some other aspects, particularly quality characteristics under varied soil types and fertility is sporadic. Nitrogen fixer and phosphate solubilizing microorganisms play an important role in supplementing nitrogen and phosphorus to plants, allowing a sustainable use of nitrogen and phosphate fertilizer" (Tambekar *et al.* 2009) [8]. The inoculation of *Rhizobium* can increase grain yield in pulse crop from 10 to 15% (Ali and Chandra, 1985) [1] while phosphate solubilizing bacteria (PSB) increase the availability of insoluble phosphorus into the soils by mineralizing organic phosphorus compounds. Soil health is defined as the continued capacity of soil to function as a living ecosystem that sustains plants, animals, and humans. Sustainable crop production depends upon maintaining favorable physical, chemical, and biological properties of soil. Continuous cultivation without balanced fertilization has led to depletion of essential nutrients and deterioration of soil quality in many agricultural regions. Integrated nutrient management involving chemical fertilizers, biofertilizers, and biological inputs has been recommended for maintaining soil fertility and ensuring long-term sustainability.

2. Materials and Methods

2.1 Experimental Location and Season

The experiment was conducted at "Students' Instructional Farm (SIF)", Department of Agronomy, Chandra Shekhar Azad University of Agriculture & Technology, Kanpur, (U.P.), which is situated between 25° 26' to 26° 58' North latitude, 79° 31' to 31° 34' East longitude and at the 125.9 meters above sea level in the alluvial tract of Indo-Gangatic Plain zone of central part of Uttar Pradesh, India during the Rabi Season 2024-25 to 2025-26.

2.2 Soil condition

The soil of experimental field was sandy loam in texture having low soil organic carbon (0.39%), low available N (185.49 kg ha⁻¹), medium available P (12.01 kg ha⁻¹) and K (141.41 kg ha⁻¹) contents with neutral to slightly alkaline pH 8.09 (1:2.5 soil: water ratio). The biological properties of the initial soil sample viz. dehydrogenase activity ranging from (0.62 to 0.65 µg TPF g⁻¹ soil day⁻¹), phosphatase enzymes (0.70 to 0.73 g PNP g⁻¹ h⁻¹ soil), urease enzymes (341.89 to 345.37 µg urea hydrolyzed g⁻¹ of soil h⁻¹) and bacterial, fungal and actinomycetes population (22.08 to 22.14 cfu x 10⁵ g⁻¹ soil), (9.97 to 10.08 cfu x 10⁴ g⁻¹ soil) and (12.10 to 12.47 cfu x 10³ g⁻¹ soil), respectively, during the 1st and 2nd year of experimental field.

2.3 Experimental Design and Treatments Details

The experiment was arranged in a Factorial Randomized Block Design (FRBD) with 54 treatment combinations involving three levels of phosphorus (control, phosphorus @ 30 kg ha⁻¹, phosphorus @ 60 kg ha⁻¹), three levels of sulphur (control, sulphur @ 20 kg ha⁻¹, sulphur @ 40 kg ha⁻¹) and two levels of bio-inoculants (control, *Rhizobium* + PSB) replicated thrice. Chickpea var. Uday (KPG-59) was taken as a test crop to observe direct effect of the treatments. The

100% recommended dose of N and K for chickpea crop was 25 kg N and 20 kg K₂O ha⁻¹, respectively. Full dose of N and K were applied as basal in the form of urea, DAP and muriate of potash manually by placement method into open furrows, respectively. The collected data were statistically analysed using standard ANOVA techniques as suggested by Gomez and Gomez (1984) [12]. The significance of treatment differences was tested at a 5% probability level. Soil samples were collected after harvesting of the crop and analysed by the standard procedure.

3. Results and Discussion

3.1 Biological properties

The data pertaining to biological properties under different treatments are presented in Table 1 and figure 1. The activity of phosphatase enzymes and dehydrogenase differed significantly amongst all the treatments. The data showed significant variation of phosphatase enzymes and soil dehydrogenase due to phosphorus, sulphur and bio-inoculants practices at amongst all the treatments during both years and on pooled basis also. Pooled analysis data of both year that, phosphatase enzymes and soil dehydrogenase was recorded significantly maximum (1.40 g PNP g⁻¹ hr⁻¹ soil) and (1.23 µg TPF g⁻¹ soil day⁻¹) under the phosphorus at @ 60 kg ha⁻¹ (P₂) followed phosphorus at @ 30 kg ha⁻¹ (P₁), (1.16 g PNP g⁻¹ hr⁻¹ soil) and (1.01 µg TPF g⁻¹ soil day⁻¹) and minimum phosphatase enzymes and soil dehydrogenase (0.90 g PNP g⁻¹ hr⁻¹ soil), (0.79 µg TPF g⁻¹ soil day⁻¹) were recorded under the treatment of control (P₀). Among the Sulphur level practices significantly maximum phosphatase enzymes and soil dehydrogenase (1.24 g PNP g⁻¹ hr⁻¹ soil) and (1.07 µg TPF g⁻¹ soil day⁻¹) during both years were recorded under sulphur @ 40 kg ha⁻¹ (S₂) followed by sulphur at @ 20 kg ha⁻¹ (S₁), (1.15 g PNP g⁻¹ hr⁻¹ soil) and (1.01 µg TPF g⁻¹ soil day⁻¹) and minimum phosphatase enzymes and soil dehydrogenase (1.07 g PNP g⁻¹ hr⁻¹ soil), (µg TPF g⁻¹ soil day⁻¹) were recorded under the treatment of control (S₀). Bio-inoculants practices also significantly influenced by application of *Rhizobium* + PSB (B₁) recorded significantly highest phosphatase enzymes and soil dehydrogenase (1.26 g PNP g⁻¹ hr⁻¹ soil) and (1.09 µg TPF g⁻¹ soil day⁻¹) as compared to without inoculation (B₀), (1.05 g PNP g⁻¹ hr⁻¹ soil) and (0.92 µg TPF g⁻¹ soil day⁻¹). The hasty decomposition of soil organic matter assures the delivery of nutrients, rendering them accessible to micro-organisms for protoplasm synthesis. In addition, the introduction of farmyard manure in combination with inorganic and bio-fertilizers might have encouraged the microbial activity to exploit the intrinsic soil organic carbon pools, which serve as substrates for dehydrogenase enzyme. In agreement with the present findings (Singh *et al.* 2018) [6], (Solangi *et al.* 2024) [7].

It is evident from data presented in Table 1, that the contents of urease enzymes (µg urea hydrolyzed g⁻¹ of soil h⁻¹) varied significantly under different treatments during both years and on pooled basis also. On the basis of pooled analysis data, it is clear that level of phosphorus significantly affected urease enzymes and application of phosphorus at @ 60 kg ha⁻¹ (P₂) recorded significantly highest urease enzymes (381.70 µg urea hydrolyzed g⁻¹ of soil h⁻¹) followed by phosphorus at @ 30 kg ha⁻¹ (P₁) (369.76 µg urea hydrolyzed g⁻¹ of soil h⁻¹) and lowest urease enzymes

(356.30 μg urea hydrolyzed g^{-1} of soil h^{-1}) was recorded under the treatment of control (P_0). Under the Sulphur level practices significantly maximum urease enzymes (373.58 μg urea hydrolyzed g^{-1} of soil h^{-1}) during both years were recorded under sulphur @ 40 kg ha^{-1} (S_2) followed by sulphur at @ 20 kg ha^{-1} (S_1), (369.21 μg urea hydrolyzed g^{-1} of soil h^{-1}) and minimum urease enzymes (364.96 μg urea hydrolyzed g^{-1} of soil h^{-1}) were recorded under the treatment of control (S_0). Bio-inoculants practices also significantly influenced by application of *Rhizobium* + PSB (B_1) recorded significantly highest urease enzymes (374.17 μg urea hydrolyzed g^{-1} of soil h^{-1}) as compared to without inoculation (B_0), (364.34 μg urea hydrolyzed g^{-1} of soil h^{-1}). The increase in microbial population and enzymatic activity with P and biofertilizer application may be associated with enhanced microbial proliferation and nutrient transformation in the soil. Similar results were reported by (Ramakrishnan *et al.* 2017) [5], (Venkateswara Rao *et al.* 2019) [9].

The data pertaining to biological properties under different treatments are presented in Table 2 and figure 2. The microbial population showed higher bacterial population as compared to actinomycetes and fungi in wheat rhizosphere after harvest of the Chickpea in both the years and on pooled basis also. The data showed that level of phosphorus practices also significantly influenced by application of phosphorus at @ 60 kg ha^{-1} (P_2) recorded significantly

highest soil bacteria, actinomycetes and fungi (24.16×10^5 cfu g^{-1} soil), (16.95×10^4 cfu g^{-1} soil) and (13.89×10^3 cfu g^{-1} soil) followed by phosphorus at @ 30 kg ha^{-1} (P_1), (23.75×10^5 cfu g^{-1} soil), (15.64×10^4 cfu g^{-1} soil), (13.04×10^3 cfu g^{-1} soil) and lowest soil bacteria, actinomycetes and fungi (22.62×10^5 cfu g^{-1} soil), (13.61×10^4 cfu g^{-1} soil) and (11.40×10^3 cfu g^{-1} soil) was recorded under the treatment of control (P_0). Level of sulphur practices also significantly influenced by application of sulphur at @ 40 kg ha^{-1} (S_2) recorded significantly highest soil bacteria, actinomycetes and fungi (23.76×10^5 cfu g^{-1} soil), (15.74×10^4 cfu g^{-1} soil) and (13.16×10^3 cfu g^{-1} soil) followed by sulphur at @ 20 kg ha^{-1} (S_1) (23.55×10^5 cfu g^{-1} soil), (15.48×10^4 cfu g^{-1} soil), (12.78×10^3 cfu g^{-1} soil) and lowest soil bacteria, actinomycetes and fungi (23.23×10^5 cfu g^{-1} soil), (14.99×10^4 cfu g^{-1} soil) and (12.39×10^3 cfu g^{-1} soil) was recorded under the treatment of control (S_0). Among Bio-inoculants practices significantly maximum soil bacteria, actinomycetes and fungi (23.81×10^5 cfu g^{-1} soil), (15.78×10^4 cfu g^{-1} soil) and (13.41×10^3 cfu g^{-1} soil) during both years were recorded under inoculation with *Rhizobium* + PSB (B_1) as compared to without inoculation (B_0), (23.21×10^5 cfu g^{-1} soil), (15.02×10^4 cfu g^{-1} soil) and (12.15×10^3 cfu g^{-1} soil). Similar findings were reported by (Gupta *et al.* 2020) [3], (Verma *et al.* 2023) [10], (Nabati *et al.* 2025) [4].

Table 1: Effect of phosphorus, sulphur and bio-inoculants on biological properties of soil

Treatments	Soil Enzymatic Activity								
	Alkaline Phosphatase (g PNP g ⁻¹ hr ⁻¹ soil)			Dehydrogenase (µg TPF g ⁻¹ soil day ⁻¹)			Urease (µg urea hydrolyzed/ g of soil/h)		
	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled
Phosphorus									
P ₀	0.89	0.92	0.90	0.77	0.80	0.79	355.12	357.48	356.30
P ₁	1.14	1.18	1.16	0.99	1.03	1.01	368.44	371.09	369.76
P ₂	1.38	1.43	1.40	1.21	1.25	1.23	380.32	383.08	381.70
SEm±	0.007	0.007	0.006	0.006	0.006	0.007	2.109	2.137	2.086
C.D at 5%	0.021	0.02	0.017	0.017	0.018	0.019	6.064	6.143	5.995
Sulphur									
S ₀	1.05	1.10	1.07	0.92	0.96	0.94	363.74	366.19	364.96
S ₁	1.13	1.18	1.15	0.99	1.04	1.01	367.90	370.53	369.21
S ₂	1.21	1.26	1.24	1.05	1.10	1.07	372.24	374.93	373.58
SEm±	0.007	0.007	0.006	0.006	0.006	0.007	2.109	2.137	2.086
C.D at 5%	0.021	0.02	0.017	0.017	0.018	0.019	6.064	6.143	5.995
Bio-inoculants									
B ₀	1.03	1.08	1.05	0.90	0.95	0.92	363.07	365.60	364.34
B ₁	1.23	1.28	1.26	1.07	1.12	1.09	372.85	375.49	374.17
SEm±	0.006	0.006	0.005	0.005	0.005	0.005	1.722	1.745	1.703
C.D at 5%	0.017	0.017	0.014	0.014	0.015	0.016	4.951	5.016	4.895

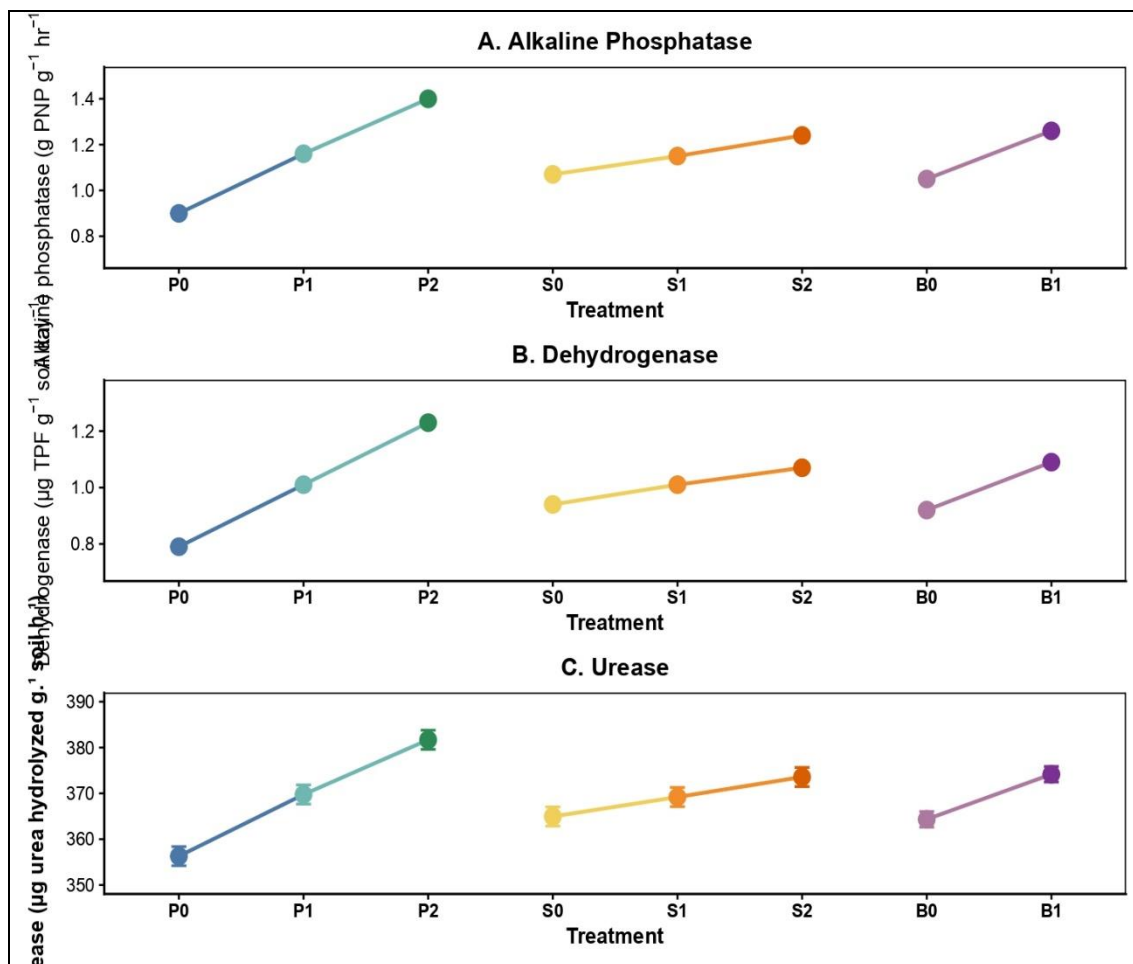


Fig 1: Effect of phosphorus, sulphur and bio-inoculants on biological properties of soil

Table 2: Effect of phosphorus, sulphur and bio-inoculants on biological properties of soil

Treatments	Microbial Population								
	Bacteria (x 10 ⁵ cfu g ⁻¹)			Actinomycetes (x 10 ⁴ cfu g ⁻¹)			Fungi (x 10 ³ cfu g ⁻¹)		
	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled	2024-25	2025-26	Pooled
Phosphorus									
P ₀	22.59	22.65	22.62	13.52	13.70	13.61	11.24	11.56	11.40
P ₁	23.73	23.77	23.75	15.56	15.72	15.64	12.92	13.15	13.04
P ₂	24.13	24.20	24.16	16.87	17.03	16.95	13.78	14.01	13.89
SEm±	0.144	0.142	0.148	0.096	0.105	0.099	0.088	0.098	0.076
C.D at 5%	0.413	0.409	0.424	0.277	0.302	0.283	0.252	0.281	0.219
Sulphur									
S ₀	23.20	23.26	23.23	14.90	15.08	14.99	12.26	12.53	12.39
S ₁	23.52	23.58	23.55	15.40	15.56	15.48	12.66	12.91	12.78
S ₂	23.73	23.78	23.76	15.66	15.82	15.74	13.03	13.29	13.16
SEm±	0.144	0.142	0.137	0.096	0.105	0.099	0.088	0.098	0.076
C.D at 5%	0.413	0.409	0.393	0.277	0.302	0.283	0.252	0.281	0.219
Bio-inoculants									
B ₀	23.19	23.24	23.21	14.94	15.11	15.02	12.03	12.27	12.15
B ₁	23.78	23.84	23.81	15.70	15.86	15.78	13.27	13.54	13.41
SEm±	0.117	0.116	0.12	0.079	0.086	0.08	0.072	0.08	0.062
C.D at 5%	0.337	0.334	0.346	0.226	0.247	0.231	0.206	0.229	0.179

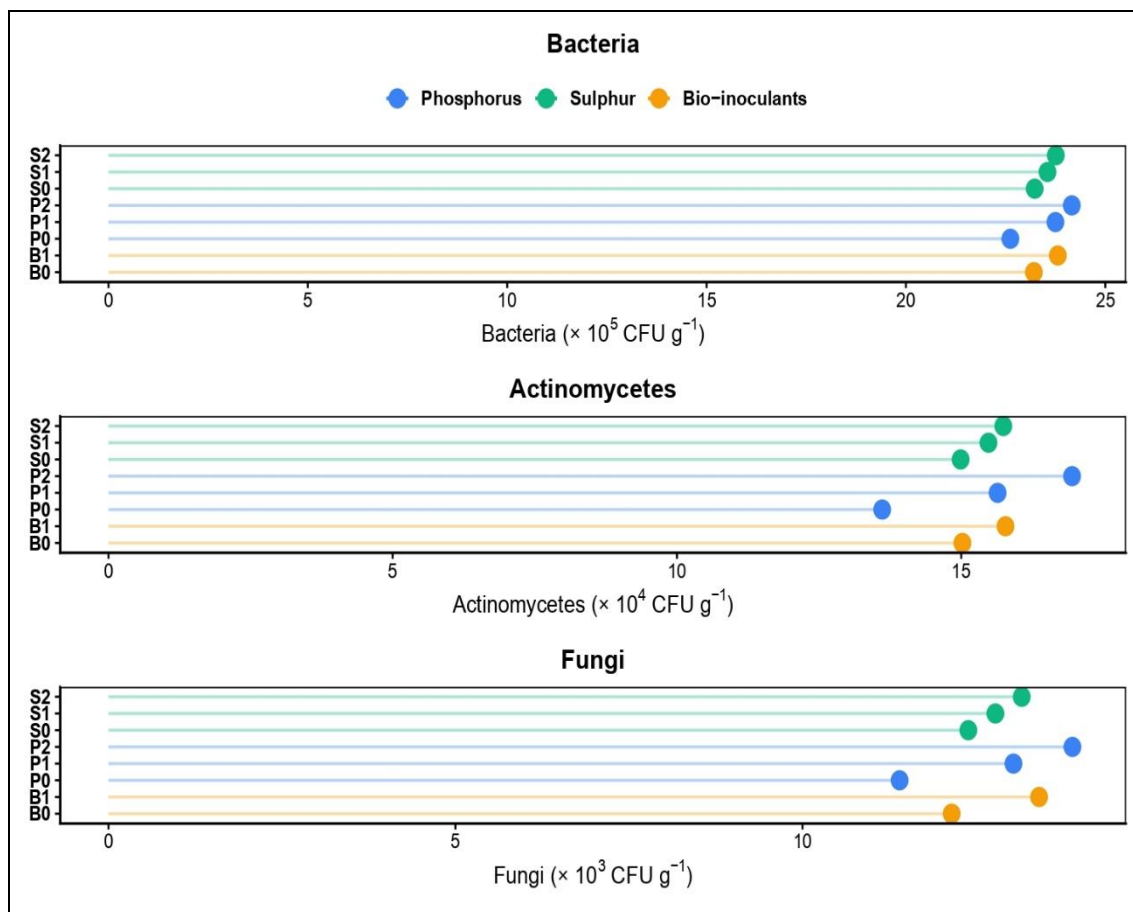


Fig 2: Effect of phosphorus, sulphur and bio-inoculants on biological properties of soil

4. Conclusion

The overall results demonstrated that enzyme activity and microbial properties of soil were improved by combined application of phosphorus, sulphur and bio-inoculants over control. Hence, it can be concluded that integration of all the treatment in balance form improve the physico-chemical and biological properties of the soil. The treatments that included phosphorus, sulphur and *Rhizobium* + PSB showed the greatest improvements in soil health, as evidenced by the increased biological count and soil enzyme activities.

5. References

1. Ali M, Chandra S. Rhizobial inoculation of pulse crops. *Indian Farming*. 1985;35(5):22-25.
2. Gomez KA, Gomez AA. *Statistical Procedures for Agricultural Research*. 2nd ed. New York: John Wiley & Sons; 1984.
3. Gupta SC, Trivedia BK, Singh P. Effect of diverse nutrient application on symbiotic traits, yield attributes, nutrient uptake, microbial population, dehydrogenase activity and productivity of chickpea (*Cicer arietinum* L.) in black soils. *Legume Research: An International Journal*. 2020;43(6):844.
4. Nabati J, Nezami A, Yousefi A, Oskoueian E, Oskoueian A, Ahmadi-Lahijani MJ. Biofertilizers containing plant growth promoting rhizobacteria enhance nutrient uptake and improve the growth and yield of chickpea plants in an arid environment. *Scientific Reports*. 2025;15(1):8331.
5. Ramakrishnan B, Kaur S, Prasanna R, Ranjan K, Kanchan A, Hossain F, *et al*. Microbial inoculation of seeds characteristically shapes the rhizosphere microbiome in desi and kabuli chickpea types. *Journal of Soils and Sediments*. 2017;17(8):2040-2053.
6. Singh G, Bhattacharyya R, Das TK, Sharma AR, Ghosh A, Das S, *et al*. Crop rotation and effects on residue soil management enzyme activities, glomalin and aggregate stability under zero tillage in the Indo-Gangetic Plains. *Soil and Tillage Research*. 2018;184:291-300.
7. Solangi F, Zhu X, Solangi KA, Iqbal R, Elsheikh MS, Alarjani KM, *et al*. Responses of soil enzymatic activities and microbial biomass phosphorus to improve nutrient accumulation abilities in leguminous species. *Scientific Reports*. 2024;14:11139.
8. Tambekar DH, Gulhane SR, Somkuwar DO, Ingle KB, Kanchalwar SP. Potential *Rhizobium* and phosphate solubilizers as biofertilizers from saline belt of Akola and Buldhana district, India. *Research Journal of Agriculture and Biological Science*. 2009;5(4):578-582.
9. Venkateswara Rao G, Sujani Rao Ch, Venkata Subbaiah P, Sree Rekha M. Effect of biogas digest on the microbial population and enzymatic activity of soil under chickpea cultivation. *The Journal of Research ANGRAU*. 2019.
10. Verma G, Yadav DD, Kumar A, Gupta G, Sharma VK, Upadhyay PK, *et al*. Effect of fertility levels and biofertilizers on the chemical and biological properties of soil under late sown chickpea (*Cicer arietinum* L.). *Annals of Agricultural Research*. 2023;40(1):57-61.