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Effect of cow-urine based bio-stimulant on microbial activity in soybean (*Glycine max*) under vertisols of Chhattisgarh

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Abstract

A field experiment entitled “Effect of Cow Urine-Based Bio-stimulant on Microbial Activity of Soybean in Vertisol of Chhattisgarh” was conducted during kharif 2023 at IGKV, Raipur, to evaluate the effect of cow urine-based bio-stimulants on soybean growth, nutrient uptake, soil fertility, and microbial activity. The experiment was laid out in Randomized Block Design with ten treatments and three replications using soybean variety CG Soya 11-15. Results revealed that treatment T₈ (75% RDF + seed soaking with 20% bio-stimulant + foliar spray of 20% bio-stimulant at 30 and 45 DAS) significantly enhanced nodulation, leghemoglobin content, nutrient uptake, soil microbial activity, and yield attributes. Maximum seed yield (18.35 q ha⁻¹) under T₈ was statistically at par with 100% RDF (19.01 q ha⁻¹). Enhanced rhizobial population, dehydrogenase activity, phosphatase activity, and microbial biomass carbon were also observed under T₈ and T₁₀ treatments. The study indicates that cow urine-based bio-stimulants can save up to 25% RDF in soybean cultivation under Vertisol conditions.

Keywords: Soybean, Cow urine-based bio-stimulant, Vertisol, Rhizobial population, Dehydrogenase activity, Phosphatase activity, Microbial biomass carbon, Nutrient uptake

Introduction

Cow urine is recognized as a valuable organic nutrient source rich in nitrogen, potassium, micronutrients, enzymes, hormones, and several bioactive compounds that promote plant growth and soil health. Unlike solid cow dung, which requires microbial decomposition before nutrient release, cow urine contains readily available nutrients such as urea, uric acid, soluble phosphates, and potassium salts that can be directly utilized by plants through foliar spray or soil application (Pradhan *et al.*, 2017) [11]. Being inexpensive, eco-friendly, and easily available to farmers through livestock systems, cow urine has gained importance in sustainable and organic agriculture.

Soybean (*Glycine max* L.) is one of the most important leguminous crops cultivated worldwide due to its high protein and oil content. Soybean seeds are rich in proteins, essential amino acids, minerals, vitamins, and unsaturated fatty acids, making them highly nutritious for human consumption (Pringgohandoko and Padmini, 1999) [12]. In Chhattisgarh, soybean is predominantly cultivated under rainfed conditions by small and marginal farmers. However, low productivity is often associated with imbalanced fertilizer use, declining soil fertility, poor microbial activity, and increasing input costs.

Cow urine-based bio-stimulants have shown considerable potential in improving nutrient availability, nutrient uptake, soil biological activity, and crop productivity. The organic compounds present in cow urine serve as substrates for soil microorganisms, thereby stimulating microbial multiplication, enzymatic activities, and nutrient mineralization. Enhanced microbial activity improves rhizosphere processes, nutrient cycling, and biological nitrogen fixation in legumes such as soybean (Hungria *et al.*, 2006) [8]. Studies have also reported that cow urine increases beneficial microbial populations and soil enzyme activities such as dehydrogenase, phosphatase, and urease, which are important indicators of soil biological health (Dinesh *et al.*, 2010) [5]. Therefore, cow urine-based bio-stimulants may provide a sustainable alternative for improving soybean productivity and maintaining soil fertility under Vertisol conditions.

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Materials and Methods

A field experiment entitled “Effect of Cow Urine-Based Bio-stimulant on Microbial Activity of Soybean in Vertisol of Chhattisgarh” was conducted during the kharif season of 2023 at the Research cum Instructional Farm of Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh. The experimental soil was Vertisol in nature. Soybean variety CG Soya 11-15 was used as the test crop. The experiment was laid out in a Randomized Block Design (RBD) with ten treatments and three replications.

The treatments consisted of different levels of recommended dose of fertilizers (RDF) combined with cow urine-based bio-stimulant applied through seed soaking, foliar spray, and

soil application. Recommended agronomic practices were followed throughout the crop period. Observations on plant growth, nodulation, yield attributes, nutrient uptake, soil nutrient status, and microbial activity were recorded at different crop growth stages.

Plant samples were analyzed for nitrogen, phosphorus, and potassium uptake using standard analytical procedures. Soil samples collected after harvest were analyzed for available nitrogen, phosphorus, potassium, microbial population, phosphatase activity, dehydrogenase activity, and microbial biomass carbon. The experimental data were statistically analyzed using analysis of variance (ANOVA) appropriate for RBD.

Table 1: Treatment Details

S. No.	Notations	Treatment details
1.	T ₁	Control (NOP0K0)
2.	T ₂	100% RDF (NPK @ 20:60:30 kg/ha)
3.	T ₃	50% RDF
4.	T ₄	75% RDF
5.	T ₅	50% RDF + Foliar spray of 20% bio-stimulant at 30 and 45 DAS
6.	T ₆	75% RDF + Foliar spray of 20% bio-stimulant at 30 and 45 DAS
7.	T ₇	50% RDF + Seed soaking through 20% bio-stimulant + Foliar spray of 20% bio-stimulant at 30 and 45 DAS
8.	T ₈	75% RDF + Seed soaking through 20% bio stimulant + Foliar spray of 20% bio-stimulant at 30 and 45 DAS
9.	T ₉	50% RDF + Soil application of 20% bio-stimulant at 10 DAS
10.	T ₁₀	75% RDF + Soil application of 20% bio-stimulant at 10 DAS

Results and Discussion

Plant Height

Plant height increased progressively with crop age, reaching maximum values at 90 DAS, with rapid growth observed between 30 and 60 DAS. Application of cow urine-based bio-stimulant along with different fertilizer levels showed no significant effect on soybean plant height compared to the control. Among treatments, T₆ (75% RDF + foliar spray

of 20% bio-stimulant at 30 and 45 DAS) recorded the highest plant height at 45 DAS (53.90 cm), 60 DAS (54.23 cm), and at harvest (54.70 cm), while the control (T₁) consistently recorded the lowest values. Overall, all treatments exhibited only non-significant increases in plant height. Similar findings were reported by Pooja *et al.*, who observed no significant influence of cow urine application on soybean plant height during early growth stages.

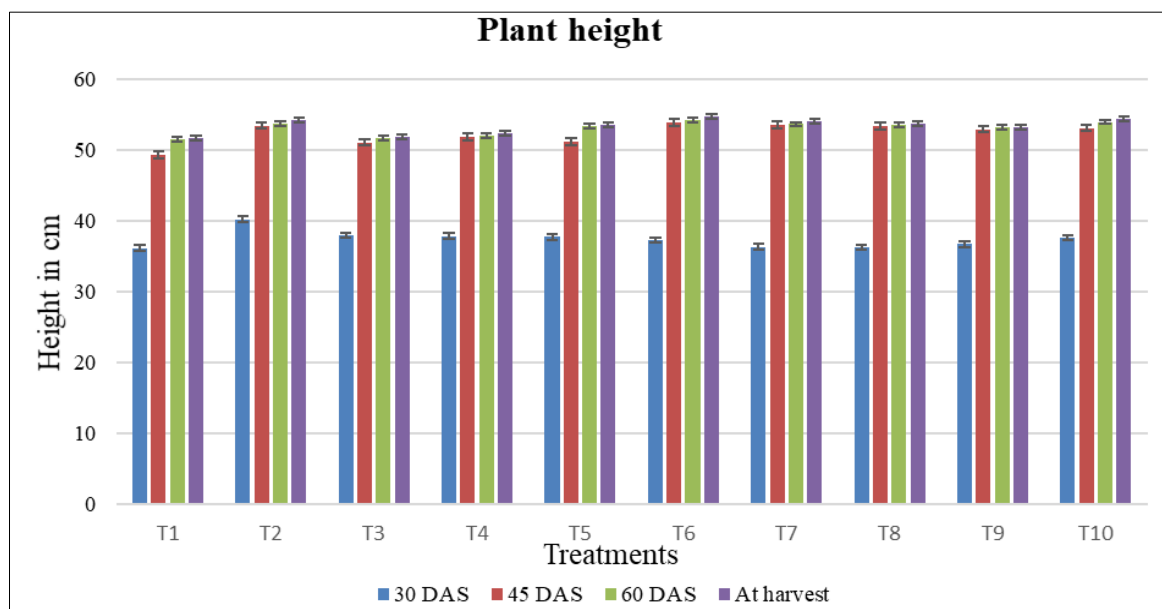


Fig 1: Effect of cow urine-based bio stimulant on plant height of soybean.

Nodulation study

At 45 DAS, the number of nodules was counted and ranged from 42.33 to 63.00 nodule/plant. Maximum number of nodules 63.00 nodule/plant was recorded with treatment T₈ (75% RDF + Seed soaking through 20% bio-stimulant +

Foliar spray of 20% bio-stimulant at 30 and 45 DAS) among all the treatment number of nodules per plant was significant as compare to the control except T₃ (50% RDF) and T₅ (50% RDF + Foliar spray of 20% bio-stimulant at 30 and 45

DAS), while the lowest number of nodules was recorded with treatment T₁ (Control) - 42.33 nodule/plant.

Nodule fresh weight was presented in Table 4.2 and Figure 4.3 indicated that highest fresh weight of nodules (1.00 g/plant) was recorded with treatment T₈ (75% RDF + Seed soaking through 20% bio-stimulant + Foliar spray of 20% bio-stimulant at 30 and 45 DAS) followed by T₆ (75% RDF + Foliar spray of 20% bio-stimulant at 30 and 45 DAS) and T₁₀ (75% RDF + Soil application of 20% bio-stimulant at 10 DAS) while lowest fresh weight of nodules was recorded in treatment T₁ (control) 0.78 g/plant.

The dry weight of nodule per plant was presented in Table 4.2 and Figure 4.3, the dry weight of nodules were ranged between 0.16 to 0.36 g/plant. The highest dry weight of nodule of soybean was associated with treatment T₈ (75% RDF + Seed soaking through 20% bio-stimulant + Foliar spray of 20% bio-stimulant at 30 and 45 DAS) 0.36g/plant as compared with T₁ (Control) 0.16g/plant. However, the values were at par of treatment T₂ (100% RDF (NPK @ 20:60:30 kg/plant)) 0.35g/plant, T₁₀ (75% RDF + Soil application of 20% bio-stimulant at 10 DAS) 0.35 g/plant and T₆ (75% RDF + Foliar spray of 20% bio-stimulant at 30 and 45 DAS) 0.34 g/plant.

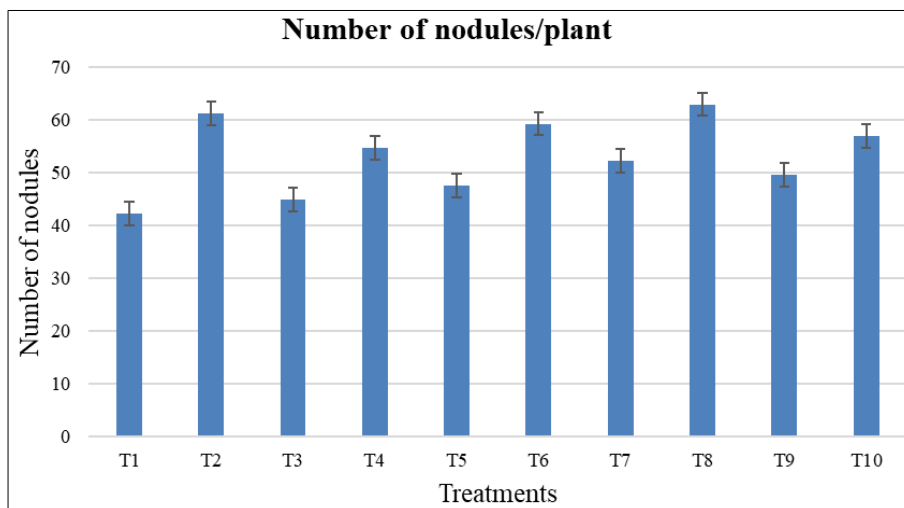


Fig 2: Effect of cow urine-based bio stimulant on nodulation of soybean crop.

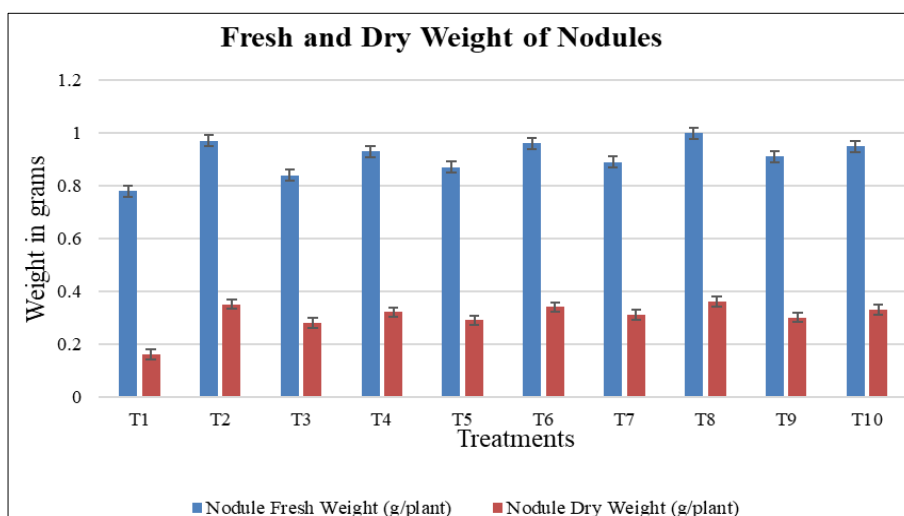


Fig 3: Effect of cow-urine based bio-stimulant on fresh and dry weight of nodules of soybean.

Test weight, Seed yield and Stover yield

Test weight indicated that the different treatments had no discernible effects. The highest test weight (104.07 g) was recorded in T₈ (75% RDF + Seed soaking through 20% bio-stimulant + Foliar spray of 20% bio-stimulant at 30 and 45 DAS), closely followed by T₆ (103.83 g) with 75% RDF + Foliar spray of 20% bio-stimulant at 30 and 45 DAS), T₁₀ (103.87 g) with 75% RDF + Soil application of 20% bio-stimulant at 10 DAS), and T₂ (101.60 g) with 100% RDF through inorganic fertilizer and treatment T₁ (Control) had the lowest test weight (94.67 g).

Soybean seed yield was significantly influenced by fertilizer levels and the application of cow urine-based bio-stimulant.

The highest seed yield was recorded with 100% RDF (19.21 q ha⁻¹), followed by T₈ (75% RDF + seed soaking and foliar spray of 20% bio-stimulant) with 18.35 q ha⁻¹, which was statistically at par with 100% RDF. Treatments involving 75% RDF combined with bio-stimulant applications also produced yields comparable to full RDF, indicating a potential saving of 25-50% chemical fertilizer without significant yield reduction. The lowest yield was recorded in the control (14.43 q ha⁻¹). The enhanced yield under integrated nutrient management may be attributed to improved soil biological activity, nutrient mobilization, uptake, and translocation promoted by the cow urine-based

bio-stimulant. Similar findings were reported by Deotale *et al.* and Gupta and Yadav.

Application of cow urine-based bio-stimulants showed no significant effect on soybean stover yield. The highest stover yield was recorded with 100% RDF (T_2) at 21.97 q ha^{-1} , while the lowest was observed in the control (T_1) at 17.21 q ha^{-1} . Although stover yield increased with increasing fertilizer levels from 50% to 100% RDF, the differences were statistically non-significant. Similar biological yield across treatments may be attributed to the medium to high availability of soil nutrients (N, P, and K) in the experimental field, along with comparable plant population and growth parameters among treatments.

Uptake of Nitrogen

Nitrogen uptake in soybean seed, stover, and total biomass was significantly influenced by the application of cow urine-based bio-stimulants and fertilizer levels. The highest seed, stover, and total nitrogen uptake were recorded with 100% RDF (T_2), registering 112.03, 60.07, and 172.10 $kg\ ha^{-1}$, respectively. Among the bio-stimulant treatments, T_8 (75% RDF + seed soaking + foliar spray of 20% bio-stimulant at 30 and 45 DAS) showed the highest nitrogen uptake and remained statistically at par with 100% RDF. All treatments significantly outperformed the control, indicating that integrated use of RDF and bio-stimulants enhanced nutrient uptake through improved plant growth and yield. Similar results were reported by Ghoya *et al.* and Shaziya *et al.*

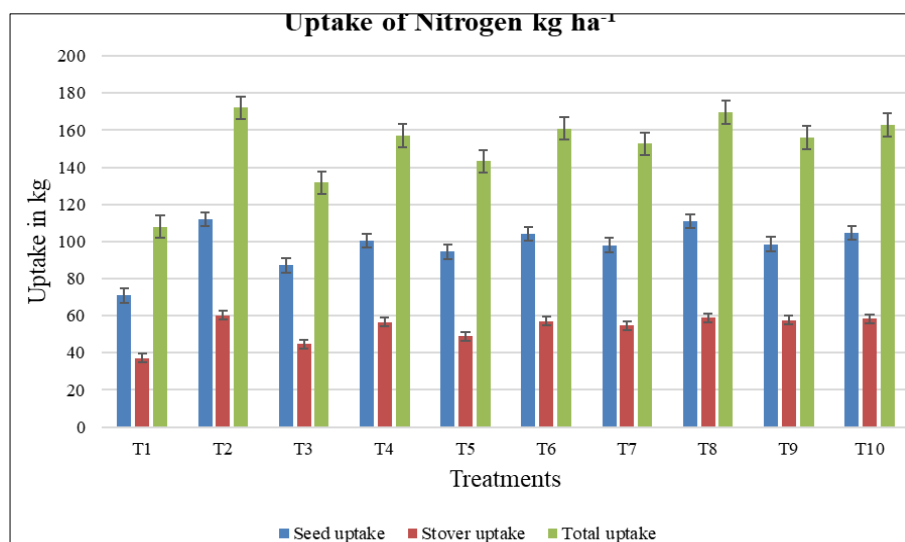


Fig 4: Effect of cow urine-based bio stimulant on uptake of nitrogen by Soybean.

Uptake of Phosphorus

Phosphorus uptake in soybean seed, stover, and total biomass was significantly influenced by the application of cow urine-based bio-stimulants and fertilizer levels. The highest total phosphorus uptake was recorded with 100% RDF (T_2) at 10.50 $kg\ ha^{-1}$, followed closely by T_8 (75% RDF + seed soaking + foliar spray of 20% bio-stimulant at 30 and 45 DAS) with 10.46 $kg\ ha^{-1}$, which remained

statistically at par with 100% RDF. All treatments significantly increased phosphorus uptake over the control (7.99 $kg\ ha^{-1}$). Among bio-stimulant treatments, T_8 showed the highest seed and total phosphorus uptake, indicating improved nutrient absorption and utilization through integrated nutrient management. Similar findings were reported by Ghoya *et al.* and Shaziya *et al.*

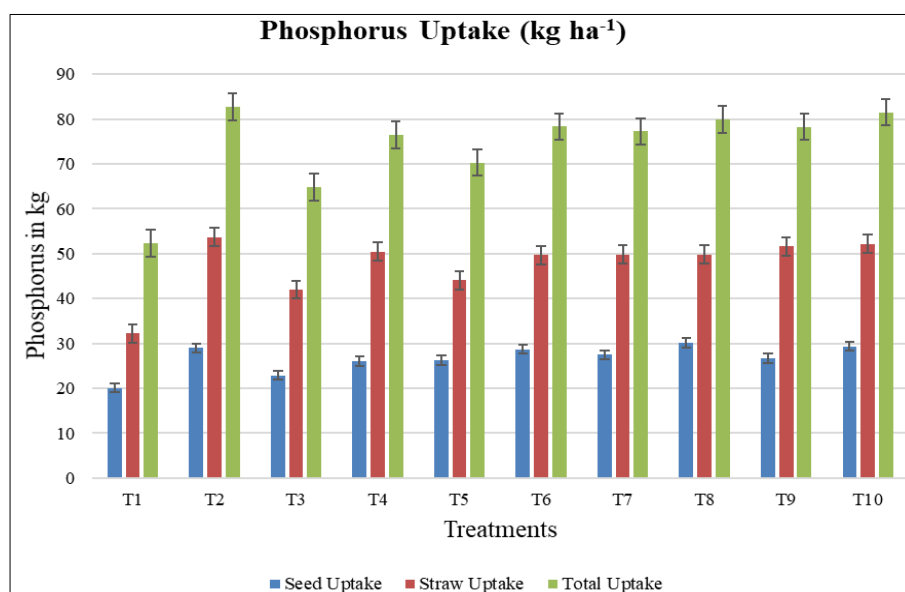


Fig 5: Effect of cow urine-based bio stimulant on Phosphorus uptake by soybean.

Uptake of Potassium

Potassium uptake in soybean seed, stover, and total biomass was significantly influenced by the application of cow urine-based bio-stimulants and fertilizer levels. The highest total potassium uptake was recorded with 100% RDF (T_2) at 82.69 kg ha^{-1} , followed closely by T_{10} (75% RDF + soil application of 20% bio-stimulant at 10 DAS) with 81.51 kg ha^{-1} and T_8 (75% RDF + seed soaking + foliar spray of 20% bio-stimulant at 30 and 45 DAS) with 79.89 kg ha^{-1} . In seed

uptake, T_8 recorded the maximum potassium uptake (30.14 kg ha^{-1}), while all treatments significantly outperformed the control (52.35 kg ha^{-1} total uptake). The enhanced potassium uptake under integrated nutrient management may be attributed to improved nutrient availability, uptake, and translocation promoted by the cow urine-based bio-stimulant. Similar findings were reported by Ghoya *et al.* and Shaziya *et al.*.

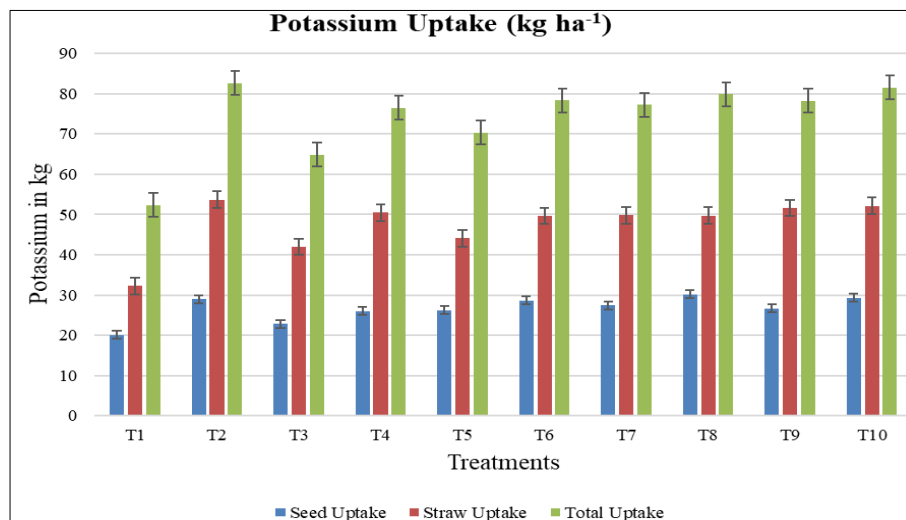


Fig 6: Effect of cow urine-based bio stimulant on potassium uptake by soybean.

Available Nitrogen, Phosphorus, Potassium

The application of cow urine-based bio-stimulants showed a non-significant effect on available soil nitrogen after soybean harvest. Soil available nitrogen increased from the initial value of $213.25 \text{ kg ha}^{-1}$ before sowing to a range of 246 kg ha^{-1} in the control (T_1) to 263.4 kg ha^{-1} in T_8 (75% RDF + seed soaking + foliar spray of 20% bio-stimulant at 30 and 45 DAS). Although differences among treatments were statistically non-significant, the overall increase in soil nitrogen after harvest may be attributed to biological nitrogen fixation by soybean. Similar findings were reported by Ghoya *et al.*.

The applied treatments significantly influenced the available phosphorus status of the soil after soybean harvest. The highest available soil phosphorus was recorded in T_2 (100% RDF) with 21.81 kg ha^{-1} , while the lowest was observed in the control (16.73 kg ha^{-1}). Among the bio-stimulant treatments with 75% RDF, T_{10} recorded comparatively

higher available phosphorus (19.89 kg ha^{-1}). Application of 50%, 75%, and 100% RDF increased soil phosphorus status, with the greatest improvement observed under 100% RDF, likely due to higher phosphorus application and partial fixation in soil. Reductions in available phosphorus in some treatments may be attributed to greater crop uptake. Similar findings were reported by Ghoya *et al.*.

The application of cow urine-based bio-stimulants had a non-significant effect on available soil potassium after soybean harvest. Available soil potassium increased from the initial value of 345.4 kg ha^{-1} before sowing to a range of 373 kg ha^{-1} in the control to 401.8 kg ha^{-1} under 100% RDF treatment. Differences among treatments were statistically non-significant, possibly due to the release of potassium from exchangeable to available forms in the soil, which maintained a balanced potassium status across treatments. Similar findings were reported by Ghoya *et al.*.

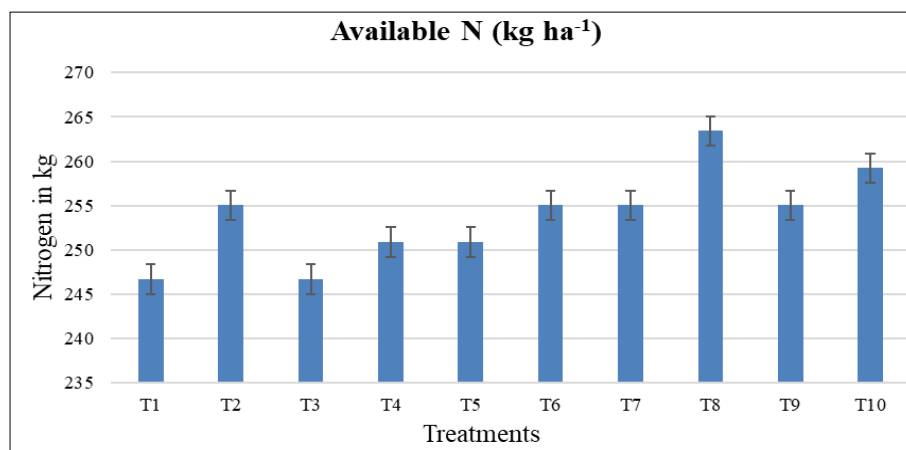


Fig 7: Effect of cow urine-based bio stimulant on soil nitrogen.

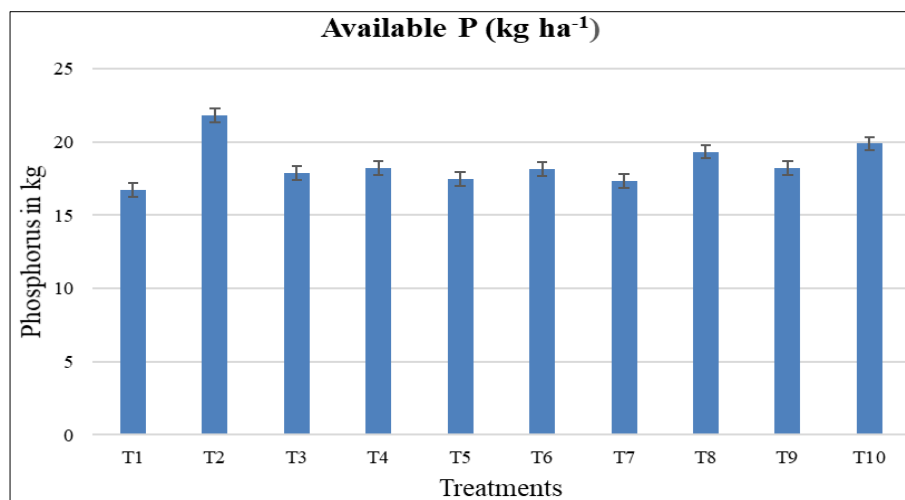


Fig 8: Effect of cow urine-based bio stimulant on soil phosphorus

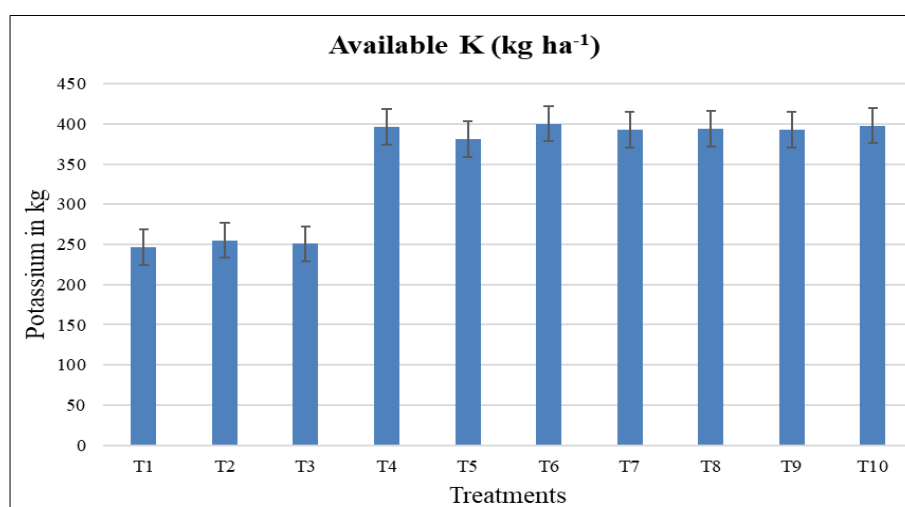


Fig 9: Effect of cow urine-based bio stimulant on soil potassium.

Soil Rhizobial Population

Soil rhizobial population density increased with crop growth and was influenced by the application of cow urine-based bio-stimulants. At 30 DAS, rhizobial population ranged from 6.176 to 6.308 log₁₀/g of rhizosphere soil, with the highest population recorded in T₁₀, followed by T₈ and T₂, while the control showed the lowest population. A similar

trend was observed at 45 DAS, where rhizobial population ranged from 6.594 to 6.768 log₁₀/g of soil, with T₁₀ again recording the maximum population and the control the minimum. The increase in microbial population may be attributed to enhanced organic activity due to cow urine-based bio-stimulant application. Similar findings were reported by Boraiah *et al.*.

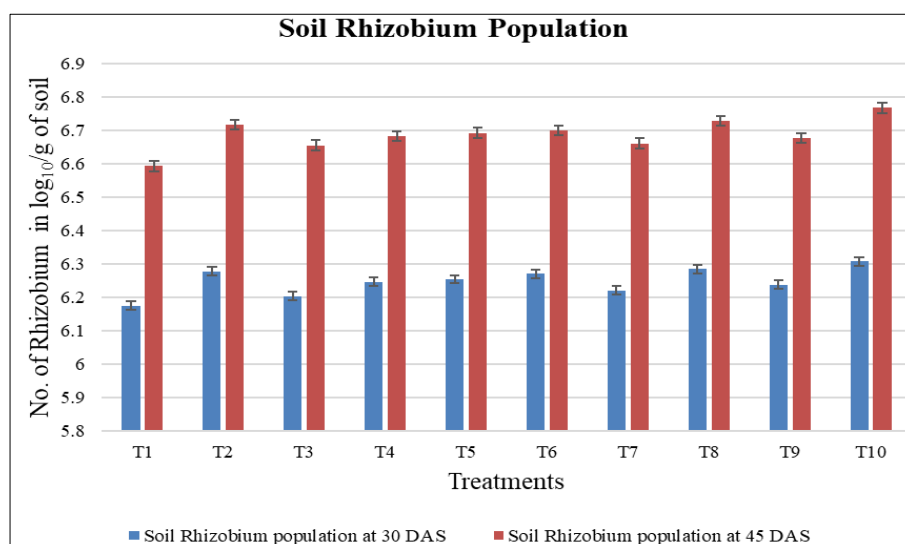


Fig 10: Effect of cow urine-based bio stimulant on soil rhizobial population

Soil Bacterial Population

The total soil bacterial population increased with the application of cow urine-based bio-stimulants throughout the crop growth period. Bacterial population density ranged from 8.486-8.672 $\log_{10}$ g⁻¹ soil at 30 DAS, 8.531-8.780 at 45 DAS, 8.564-8.799 at 60 DAS, and 8.431-8.616 after harvest. Treatments T₈ (75% RDF + seed soaking + foliar spray of

20% bio-stimulant) and T₁₀ (75% RDF + soil application of 20% bio-stimulant at 10 DAS) consistently recorded the highest bacterial populations, while the control (T₁) recorded the lowest values at all stages. The increase in bacterial population may be attributed to enhanced microbial activity promoted by cow urine-based bio-stimulants. Similar findings were reported by Veerasha *et al.*

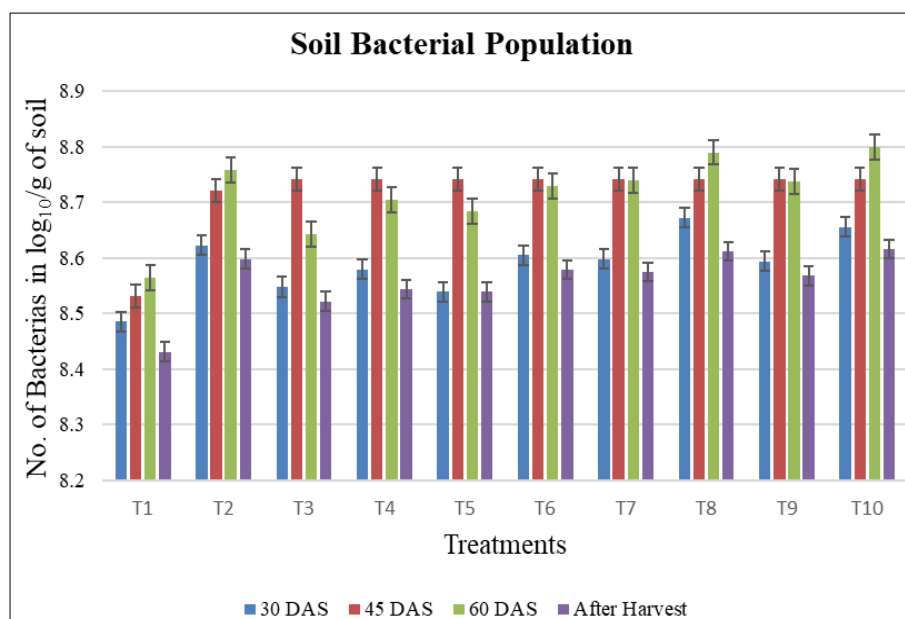


Fig 11: Effect of cow urine-based bio stimulant on soil bacterial population.

Dehydrogenase Activity

Application of cow urine-based bio-stimulants significantly increased soil dehydrogenase activity (DHA) compared to the control. At 60 DAS, the highest DHA was recorded in T₈ (75% RDF + seed soaking + foliar spray of 20% bio-stimulant) with 12.98 TPF g⁻¹ hr⁻¹ soil, followed by T₁₀ and T₂, while the control recorded the lowest activity (8.20 TPF

g⁻¹ hr⁻¹ soil). A similar trend was observed after harvest, where T₈ maintained the highest DHA (12.00 TPF g⁻¹ hr⁻¹ soil) and the control the lowest (6.89 TPF g⁻¹ hr⁻¹ soil). Increased DHA indicates enhanced microbial activity due to bio-stimulant application, though enzyme activity declined after harvest. Similar findings were reported by Bertram and Rao *et al.*

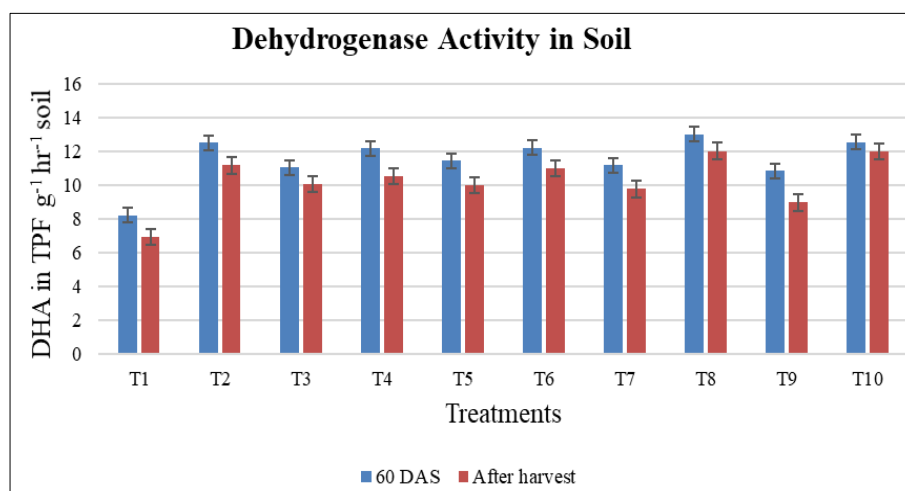


Fig 12: Effect of cow urine-based bio stimulant on dehydrogenase activity.

Phosphatase Activity:

Acid phosphatase activity in soybean rhizosphere soil was significantly influenced by different bio-stimulant treatments. At 60 DAS, the highest activity was recorded in T₈ (75% RDF + seed soaking + foliar spray of 20% bio-stimulant) with 15.20 μ g PNP g⁻¹ soil hr⁻¹, followed by T₁₀ (14.86) and T₂ (11.98), while the lowest activity was

observed in T₁ (control) with 8.28 μ g PNP g⁻¹ soil hr⁻¹. After harvest, maximum phosphatase activity was recorded in T₆ (12.36), closely followed by T₁₀ (12.20), T₈ (12.18), and T₂ (12.05), whereas the minimum was again found in T₁ (8.08 μ g PNP g⁻¹ soil hr⁻¹). The increased phosphatase activity was attributed to enhanced microbial activity due to the application of cow urine-based bio-stimulant.

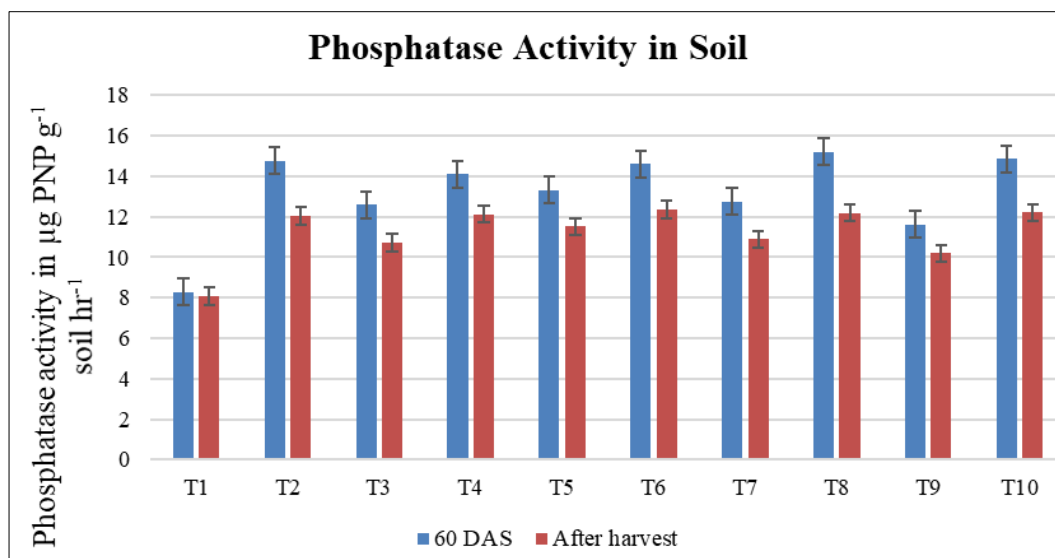


Fig 13: Effect of cow urine-based bio stimulant on soil phosphatase activity.

Soil Microbial Biomass Carbon:

Microbial biomass carbon in soybean rhizosphere soil increased significantly with the application of cow urine-based bio-stimulants. At 60 DAS, the highest microbial biomass carbon was recorded in T₈ (75% RDF + seed soaking + foliar spray of 20% bio-stimulant) with 21.64 µg C g⁻¹ soil, followed by T₁₀ (20.59), T₄ (18.39), and T₆ (17.99), while the lowest value was observed in T₁ (control)

with 9.35 µg C g⁻¹ soil. After harvest, T₈ again recorded the maximum value (19.05 µg C g⁻¹ soil), followed by T₁₀ (17.96), T₆ (16.11), and T₄ (16.00), whereas the minimum was recorded in T₁ (6.20). The increase in microbial biomass carbon was attributed to enhanced microbial activity due to the application of cow urine-based bio-stimulants, which was also supported by findings of Chaudhari *et al.* and Koirala *et al.*

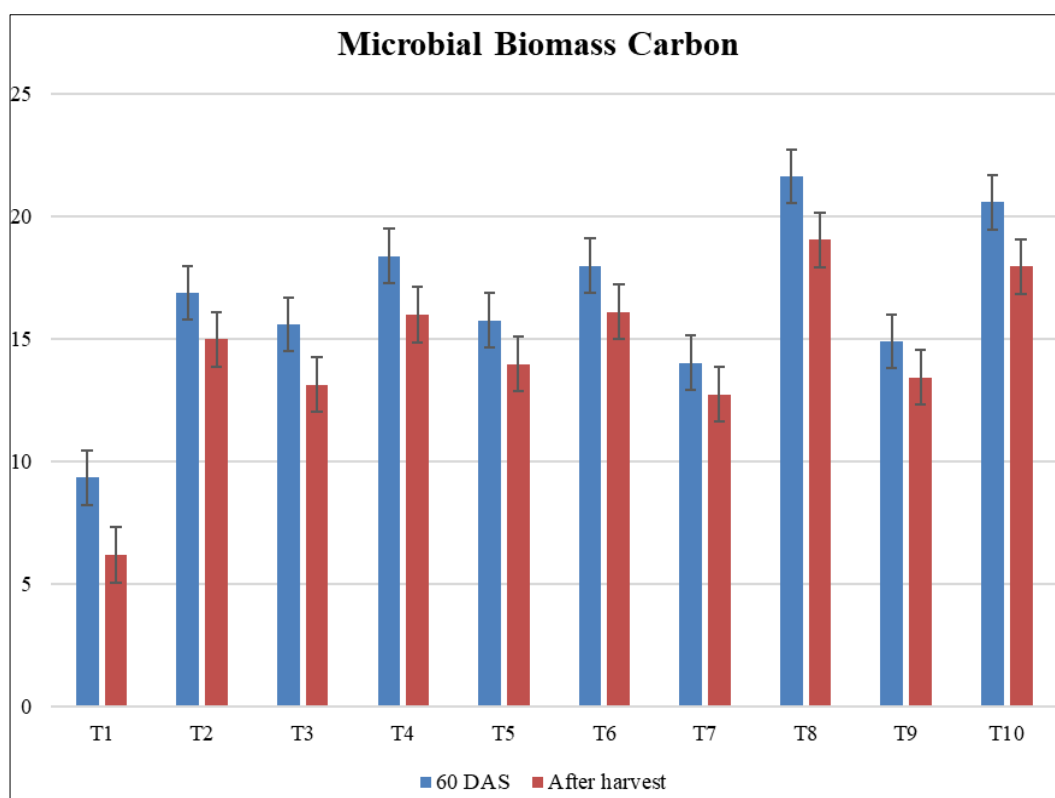


Fig 14: Effect of cow urine-based bio stimulant on microbial biomass carbon.

Table 1: Effect of Cow-Urine Based Bio-Stimulant on Growth Parameters, Nodulation and Yield Attributes of Soybean

Treatment	Treatment Details	Plant Height (cm)				Nodulation (at 45 DAS)				Yield & Yield Attributes		
		30 DAS	45 DAS	60 DAS	At Harvest (cm)	No. of Nodules plant ⁻¹	Fresh Wt. (g plant ⁻¹)	Dry Wt. (g plant ⁻¹)	Leghb. Content (mg g ⁻¹ fresh wt.)	Seed Yield (q ha ⁻¹)	Stover Yield (q ha ⁻¹)	Test Weight (g)
T ₁	Control (NoPoKo)	36.2	49.38	51.55	51.74	42.33	0.78	0.16	1.45	14.43d	17.21	94.67
T ₂	100% RDF (NPK @ 20:60:30)	40.27	53.49	53.76	54.3	61.33	0.97	0.35	3.05	19.01a	21.97	101.6
T ₃	50% RDF	37.96	51.09	51.68	51.9	45	0.84	0.28	2.6	15.21cd	17.59	95.17
T ₄	75% RDF	37.9	51.89	52.1	52.42	54.67	0.93	0.32	2.85	17.17abc	20.98	96.77
T ₅	50% RDF + Foliar spray (20%) 30 & 45 DAS	37.75	53.21	53.45	53.55	47.67	0.87	0.29	2.15	16.15bc	18.3	95.43
T ₆	75% RDF + Foliar spray (20%) 30 & 45 DAS	37.29	53.9	54.23	54.7	59.33	0.96	0.34	2.95	17.68abc	20.55	103.83
T ₇	50% RDF + Seed soaking (20%) + Foliar spray (20%) 30 & 45 DAS	36.37	53.61	53.7	54.05	52.33	0.89	0.31	2.65	16.77abc	20.48	102.5
T ₈	75% RDF + Seed soaking (20%) + Foliar spray (20%) 30 & 45 DAS	36.31	53.45	53.64	53.75	63	1	0.36	3.25	18.35ab	21.17	104.07
T ₉	50% RDF + Soil application (20%) at 10 DAS	36.73	52.99	53.29	53.3	49.67	0.91	0.3	2.55	16.46bcd	21.11	95.73
T ₁₀	75% RDF + Soil application (20%) at 10 DAS	37.68	53.15	54	54.43	57	0.95	0.33	3	17.85abc	21.22	103.87
SEm ±		0.705	0.258	0.173	0.161	1.630	0.020	0.011	0.0986	0.78	NS	NS
CD (5%)		2.173	0.795	0.532	0.498	5.325	0.061	0.033	0.383	2.32	NS	NS

RDF = Recommended Dose of Fertilizer (NPK @ 20:60:30 kg ha⁻¹); DAS = Days after sowing; Leghb. = Leghemoglobin; NS = Non-Significant; Values in parentheses indicate significance grouping at 5% level.

Table 2: Effect of Cow-Urine Based Bio-Stimulant on Nutrient Uptake by Soybean and Post-Harvest Soil Macronutrient Status

Treatment	Treatment Details	N Uptake (kg ha ⁻¹)			P Uptake (kg ha ⁻¹)			K Uptake (kg ha ⁻¹)			Post-Harvest Available Soil Nutrients		
		Seed	Stover	Total	Seed	Stover	Total	Seed	Stover	Total	Avail. N (kg ha ⁻¹)	Avail. P (kg ha ⁻¹)	Avail. K (kg ha ⁻¹)
T ₁	Control (NoPoKo)	70.92	37.09	108.01	5.18	2.76	7.99	20.09	32.26	52.35	246.7	16.73e	373.81
T ₂	100% RDF (NPK @ 20:60:30)	112.03	60.07	172.1	6.74	3.76	10.5	29.01	53.68	82.69	255.06	21.81a	401.86
T ₃	50% RDF	87.14	44.66	131.79	5.24	2.88	8.05	22.86	41.98	64.84	246.7	17.91d	383.56
T ₄	75% RDF	100.55	56.56	157.11	6.21	3.81	10.02	26.04	50.44	76.48	250.88	18.20cd	396.18
T ₅	50% RDF + Foliar spray (20%) 30 & 45 DAS	94.42	48.88	143.3	5.48	2.9	8.39	26.24	44.08	70.31	250.88	17.47de	381.29
T ₆	75% RDF + Foliar spray (20%) 30 & 45 DAS	104.02	56.92	160.94	6.3	3.73	10.03	28.67	49.66	78.33	255.06	18.17cd	400.29
T ₇	50% RDF + Seed soaking (20%) + Foliar spray (20%) 30 & 45 DAS	98.08	54.64	152.72	6.2	3.77	9.96	27.45	49.83	77.28	255.06	17.33de	392.97
T ₈	75% RDF + Seed soaking (20%) + Foliar spray (20%) 30 & 45 DAS	110.84	58.83	169.67	6.6	3.86	10.46	30.14	49.75	79.89	263.42	19.34bc	393.95
T ₉	50% RDF + Soil application (20%) at 10 DAS	98.49	57.5	155.99	5.95	3.8	9.75	26.66	51.6	78.26	255.06	18.25cd	392.95
T ₁₀	75% RDF + Soil application (20%) at 10 DAS	104.53	58.34	162.87	6.22	3.81	10.03	29.33	52.18	81.51	259.24	19.89b	397.89
SEm ±		4.42	2.40	5.23	0.35	0.27	0.56	1.78	2.74	4.0	NS	0.39	NS
CD (5%)		12.09	6.09	15.12	1.05	0.79	1.67	5.27	8.13	11.9	NS	1.17	NS

N = Nitrogen; P = Phosphorus; K = Potassium; Avail. = Available; NS = Non-Significant. Superscript letters denote significant differences at 5% level (Duncan's test).

Table 3: Effect of Cow-Urine Based Bio-Stimulant on Soil Microbial Activity — Population Dynamics and Enzyme Activities

Treatment	Treatment Details	Rhizobium Population (Log ₁₀ g ⁻¹ rhizosphere soil)		Total Bacterial Population (Log ₁₀ g ⁻¹ soil)				Dehydrogenase Activity (µg TPF g ⁻¹ soil hr ⁻¹)		Phosphatase Activity (µg PNP g ⁻¹ soil hr ⁻¹)		Microbial Biomass Carbon (µg C g ⁻¹ soil)	
		30 DAS	45 DAS	30 DAS	45 DAS	60 DAS	After Harvest	60 DAS	After Harvest	60 DAS	After Harvest	60 DAS	After Harvest
T ₁	Control (NoPoKo)	6.176	5.594	8.486	8.531	8.564	8.431	8.2	6.89	8.28	8.08	9.35	6.2
T ₂	100% RDF (NPK @ 20:60:30)	6.278	6.718	8.623	8.721	8.758	8.598	12.47	11.14	14.76	12.05	16.9	15
T ₃	50% RDF	6.204	6.656	8.548	8.598	8.643	8.522	11.02	10.02	12.6	10.72	14.92	13.14
T ₄	75% RDF	6.247	6.684	8.579	8.646	8.704	8.544	12.15	10.5	14.1	12.12	18.39	16
T ₅	50% RDF + Foliar spray (20%) 30 & 45 DAS	6.255	6.693	8.539	8.623	8.684	8.539	11.42	9.98	13.32	11.52	15.61	13.99
T ₆	75% RDF + Foliar spray (20%) 30 & 45 DAS	6.271	6.701	8.605	8.698	8.729	8.579	12.19	10.97	14.6	12.36	17.99	16.11
T ₇	50% RDF + Seed soaking (20%) + Foliar spray (20%) 30 & 45 DAS	6.221	6.662	8.598	8.675	8.74	8.575	11.16	9.74	12.74	10.88	16.12	14.21
T ₈	75% RDF + Seed soaking (20%) + Foliar spray (20%) 30 & 45 DAS	6.286	6.729	8.672	8.78	8.79	8.612	12.98	12	15.2	12.18	21.64	19.05
T ₉	50% RDF + Soil application (20%) at 10 DAS	6.238	6.678	8.594	8.662	8.737	8.568	10.82	8.94	11.6	10.2	15.77	15.61
T ₁₀	75% RDF + Soil application (20%) at 10 DAS	6.308	6.768	8.656	8.742	8.799	8.616	12.51	11.98	14.86	12.2	20.59	17.96
SEm ±		0.120	0.373	0.319	0.361	0.452	0.354	0.378	0.479	0.519	0.545	0.413	0.675
CD (5%)		0.370	0.623	0.910	0.960	1.010	0.946	1.240	1.186	1.598	1.680	1.293	2.081

TPF = Triphenyl Formazan; PNP = p-Nitrophenol; MBC = Microbial Biomass Carbon. All microbial populations expressed as Log₁₀ colony forming units g⁻¹ soil. Measurements at 60 DAS (rhizosphere soil) and after-harvest (bulk soil).

Conclusion

The experiment concluded that application of cow urine-based bio-stimulant significantly improved soybean yield, soil microbial population, rhizobial population, dehydrogenase activity, phosphatase activity, and microbial biomass carbon. Among all treatments, 75% RDF + seed soaking with 20% bio-stimulant + foliar spray of 20% bio-stimulant at 30 and 45 DAS proved most effective. This treatment recorded the highest seed yield (18.35 q ha⁻¹), which was statistically comparable to 100% RDF (19.01 q ha⁻¹), indicating a saving of 25% RDF. Nutrient uptake (N, P, and K) under 50-75% RDF with bio-stimulant was also comparable to 100% RDF. Soil available phosphorus after harvest was highest under 100% RDF application. Overall, bio-stimulant application enhanced crop productivity and soil biological health while reducing fertilizer requirement.

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