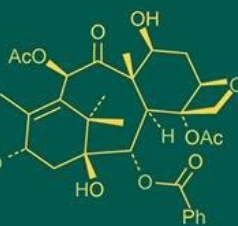
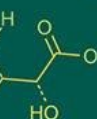


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Soil biological responses to *Rhizobium* and Rhizobacteria inoculation in green gram cultivation

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Abstract

Soil health is a critical determinant of sustainable pulse production, particularly in intensive cropping systems where biological activity and organic carbon often decline over time. The present study evaluated the influence of *Rhizobium* strains and rhizobacterial consortia on soil biological and chemical properties in green gram (*Vigna radiata* L.) varieties DGGV-2 and DGG-96. Field experiments were conducted using individual inoculants and multi-strain consortia, and soil dehydrogenase activity, organic carbon, and organic matter were assessed at different crop growth stages. Soil dehydrogenase activity, a sensitive indicator of microbial metabolic activity, was significantly enhanced by microbial inoculation, with the consortium treatment comprising *Rhizobium* strains HR-73 and BR-29 along with AUDP 203 and AUDP 279 recording the highest activity across 30, 45, and 60 days after sowing. Maximum enzyme activity was observed at 45 DAS, coinciding with peak flowering and pod development stages. Similarly, soil organic carbon and organic matter content showed significant improvement under consortium treatments compared to uninoculated controls, indicating improved microbial colonization and nutrient mobilization in the rhizosphere. Among the two varieties, DGGV-2 consistently exhibited superior soil biological activity and carbon enrichment compared to DGG-96. The interaction between crop variety and microbial consortium was statistically significant, highlighting the importance of host-microbe compatibility. Overall, the study demonstrates that application of well-designed microbial consortia can substantially enhance soil health parameters in green gram, offering a sustainable alternative to sole reliance on chemical inputs.

Keywords: Green gram, microbial inoculum, *Rhizobium*, Soil dehydrogenase activity, soil organic carbon

Introduction

Sustainable pulse production is increasingly challenged by declining soil fertility, reduced microbial activity, and depletion of soil organic carbon due to continuous cultivation and excessive dependence on chemical fertilizers. Green gram (*Vigna radiata* L.) is an important pulse crop valued for its high protein content, short duration, and ability to improve soil fertility through biological nitrogen fixation. However, the full potential of green gram in enhancing soil health can only be realized when supported by an active and diverse soil microbial community.

Soil microorganisms play a pivotal role in nutrient cycling, organic matter decomposition, and maintenance of soil structure. Among the various biological indicators of soil health, soil enzyme activities, particularly dehydrogenase activity, are considered reliable measures of overall microbial metabolic activity. Dehydrogenase enzymes are present in all living microbial cells and reflect the intensity of oxidative processes occurring in the soil. Hence, changes in dehydrogenase activity directly indicate variations in soil biological status resulting from management practices.

Rhizobium inoculation has long been recognized for its role in symbiotic nitrogen fixation in legumes. In recent years, the use of rhizobacterial consortia combining *Rhizobium* with other beneficial plant growth-promoting rhizobacteria has gained attention due to their synergistic effects on plant growth and soil quality. Such consortia not only enhance nitrogen fixation but also improve nutrient solubilization, organic matter turnover, and microbial biomass in the rhizosphere.

Soil organic carbon is another key indicator of soil health, influencing nutrient availability, water retention, and microbial activity.

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Enhancement of soil organic carbon through biological means is particularly desirable for sustainable agriculture, as it contributes to long-term soil productivity and resilience. Despite the recognized benefits of microbial inoculants, information on their combined effects on soil enzyme activity and organic carbon dynamics in green gram varieties remains limited.

Therefore, the present study was undertaken to evaluate the effect of different *Rhizobium* strains and rhizobacterial consortia on soil dehydrogenase activity, soil organic carbon, and organic matter content in two green gram varieties under greenhouse conditions. The treatment used for the study were characterized earlier for plant growth promoting traits. The study also aimed to understand the interaction between crop variety and microbial inoculation in improving soil health parameters under field conditions.

Materials and Methods

Soil enzyme activity (Dehydrogenase activity)

Soil dehydrogenase activity was estimated at 30, 45 and 60 DAS. Ten grams of soil and 0.2 g CaCO₃ were thoroughly mixed and dispensed in test tubes. To each tube, one ml of 3 percent aqueous solution of 2, 3, 5-Triphenyl tetrazolium chloride (TTC), one ml of one percent glucose solution and eight ml of distilled water were added. A thin film of water above the soil layer helped to create anaerobic condition. The tubes were stoppered with rubber cork and incubated at 28±2°C for 24 hr in dark. At the end of incubation, the contents of the tube were rinsed down into a small beaker and slurry was made by adding 10 ml methanol. The slurry was filtered through Whatman No. 42 filter paper. Repeated rinsing of soil with one ml methanol was continued till the filtrate ran free of orange colour. The filtrate was pooled and made up to 50 ml with methanol in a volumetric flask. The intensity of red colour was measured at 485 nm against methanol as blank using spectrophotometer. The concentration of formazan in soil samples was determined by referring to a standard curve prepared using graded concentration of formazan. The results were expressed as µg of triphenyl formazan (TPF) formed per gram soil per day (Casida *et al.*, 1964) [3].

$$\text{Conversion factor} = \frac{\text{Optical density (OD) at 485 nm}}{0.039} \times 100$$

Determination of Soil organic carbon

Soil organic carbon was estimated at 45 and 60 DAS. The

soil organic carbon (SOC) was determined by following the wet oxidation method of Walkley and Black (1934) [4] and soil organic matter is estimated using conversion factor of SOC X 1.724. Two gram of two mm sieved soil sample was ground in a poule and mortar and passed through a 0.2 mm sieve. One gram of this sample was transferred into a 500 ml dry conical flask. To this, 10 ml of 0.1 N K₂Cr₂O₇ was added and mixed well. Then, 20 ml of conc. H₂SO₄ was added and the flasks were swirled for 2-3 min and allowed to stand for 30 min on an asbestos sheet. Two hundred ml of distilled water was poured to dilute the suspension. Ten ml of 85 percent H₃PO₄ and one ml (10 drops) of diphenylamine indicator were added, which imparted violet color to the suspension. The contents of the flask were titrated against 0.5 N ferrous ammonium sulfate until the color changed from violet through dark blue to bright green. A blank reading was also taken without soil. The percent organic carbon in the soil was calculated by using the formula.

$$\text{Organic matter (\%)} = \{N \text{ of FAS} \times (\text{BLV} - \text{STV}) \times 0.003 \times 100\} \times 1.33 / \text{Weight of soil sample (g)}$$

Where, N = Normality; STV = Sample Titrated Value; BTV = Blank Titrated Value; FAS = Ferrous Ammonium Sulphate

Results

Dehydrogenase activities (µg TPF formed g⁻¹ soil d⁻¹)

Dehydrogenase activity of soil from all treatments were recorded at 30, 45 and 60 DAS. The data was presented in Table 1. Inoculation of consortium for green gram varieties, increased the dehydrogenase activity of green gram at harvest when compared to controls.

Among all observation recorded at different DAS *i.e.*, 30, 45 and 60 the treatment M7 inoculum (44.17, 86.46, 47.59) shown highest dehydrogenase activity in µg TPF formed g⁻¹ soil d⁻¹, when compared to other treatments followed by M5 inoculum (39.87, 76.16, 46.57), M6 inoculum (31.12, 65.06, 45.24) and least dehydrogenase activity among all treatments was shown by M9 inoculum (23.42, 44.81, 32.16) whereas, M1 inoculum with NC-92 showed dehydrogenase activity of 25.84, 48.74, 36.69. Among the varieties, DGGV-2 with an average of 32.28, 61.39, 41.37 shown higher dehydrogenase activity in terms of in µg TPF formed g⁻¹ soil d⁻¹ when compared to DGG-96 (29.59, 57.55, 39.56) at 30, 45 and 60 DAS respectively

Table 1: Influence of *Rhizobium* and rhizobacteria on soil dehydrogenase activity in green gram varieties DGGV-2 and DGG-96

Treatments	Dehydrogenase activity (µg TPF formed g ⁻¹ soil d ⁻¹)								
	30 DAS			45 DAS			60 DAS		
Microbial inoculants	DGGV-2	DGG-96	Mean values	DGGV-2	DGG-96	Mean values	DGGV-2	DGG-96	Mean values
M1: NC-92 (Reference strain)	28.36	23.33	25.85	51.44	46.03	48.74	37.28	36.10	36.69
M2: HR-73	29.50	29.87	29.69	55.26	47.63	51.45	37.05	37.95	37.50
M3: BR-29	26.15	28.24	27.20	51.18	44.20	47.69	34.23	34.52	34.38
M4: HR-73+ BR-29	30.77	26.15	28.46	59.06	48.73	53.90	40.15	35.47	37.81
M5: HR-73+ AUDP 203+ AUDP 279	36.15	43.59	39.87	74.91	77.41	76.16	47.38	45.76	46.57
M6: BR-29+ AUDP 203 + AUDP 279	33.97	28.27	31.12	64.11	66.00	65.06	46.15	44.32	45.24
M7: HR-73+ BR-29+ AUDP 203 + AUDP 279	44.23	44.10	44.17	87.19	85.73	86.46	48.51	46.67	47.59
M8: NC-92 + AUDP 203 + AUDP 279	35.77	23.85	29.81	60.65	63.06	61.86	45.63	42.53	44.08
M9: UIC	24.40	22.44	23.42	49.49	40.13	44.81	33.98	30.34	32.16
M10: HR-73 + BR-29 + NC-92	33.46	26.10	29.78	60.64	56.56	58.60	43.37	41.92	42.65
Mean of variety	32.28	29.59		61.39	57.55		41.37	39.56	
Comparison of	S. Em. (±)		CD (p = 0.01)	S. Em. (±)		CD (p = 0.01)	S. Em. (±)		CD (p = 0.01)
V (Variety)	0.01		0.04	0.02		0.06	0.01		0.03
R (Isolates and consortia)	0.03		0.08	0.05		0.13	0.03		0.07

Interaction V*R (Variety *Isolates)	0.04	0.12	0.06	0.19	0.04	0.10
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Table 2: Influence of *Rhizobium* and rhizobacteria on soil organic carbon in green gram varieties DGGV-2 and DGG-96

Treatments	Organic carbon (%)					
	45 DAS			60 DAS		
Microbial inoculants	DGGV-2	DGG-96	Mean values	DGGV-2	DGG-96	Mean values
M1: NC-92 (Reference strain)	0.87	1.95	1.41	0.54	0.69	0.61
M2: HR-73	0.90	2.13	1.51	1.20	0.72	0.96
M3: BR-29	0.84	1.67	1.26	0.42	0.48	0.45
M4: HR-73+ BR-29	1.77	2.37	2.07	1.23	0.93	1.08
M5: HR-73+ AUDP 203+ AUDP 279	4.17	3.27	3.72	2.31	1.62	1.96
M6: BR-29+ AUDP 203 + AUDP 279	1.92	2.67	2.29	1.74	1.05	1.39
M7: HR-73+ BR-29+ AUDP 203 + AUDP 279	5.43	3.27	4.35	4.20	1.80	3.00
M8: NC-92 + AUDP 203 + AUDP 279	2.67	2.82	2.74	1.80	1.47	1.63
M9: UIC	0.60	1.45	1.02	0.03	0.36	0.19
M10: HR-73 + BR-29 + NC-92	1.89	2.40	2.14	1.50	1.05	1.27
Mean of variety	2.106	2.40067		1.50	1.02	
	Sem (+)	CD (p = 0.01)		Sem (+)	CD (p = 0.01)	
C.D of V (Variety)	0.00024	0.0009		0.00024	0.0009	
C.D of R (Isolates)	0.00053	0.00202		0.00053	0.00202	
C.D of V*R (Variety *Isolates)	0.00075	0.00286		0.00075	0.00286	

Table 3: Influence of *Rhizobium* and rhizobacteria on soil organic matter in green gram varieties DGGV-2 and DGG-96

Treatments	Organic matter (%)					
	45 DAS			60 DAS		
Microbial inoculants	DGGV-2	DGG-96	Mean values	DGGV-2	DGG-96	Mean values
M1: NC-92 (Reference strain)	1.50	2.90	2.20	1.05	1.40	1.22
M2: HR-73	1.55	3.37	2.46	1.60	1.19	1.39
M3: BR-29	1.45	2.90	2.17	0.93	0.83	0.88
M4: HR-73+ BR-29	3.05	3.68	3.36	2.07	1.24	1.65
M5: HR-73+ AUDP 203+ AUDP 279	7.22	5.64	6.43	4.00	3.00	3.50
M6: BR-29+ AUDP 203 + AUDP 279	3.30	4.60	3.95	2.59	2.53	2.57
M7: HR-73+ BR-29+ AUDP 203 + AUDP 279	9.37	5.64	7.50	7.24	3.13	5.18
M8: NC-92 + AUDP 203 + AUDP 279	4.60	4.88	4.74	3.10	2.80	2.95
M9: UIC	1.34	0.67	1.00	0.05	0.62	0.33
M10: HR-73 + BR-29 + NC-92	3.26	4.08	3.67	2.12	1.81	1.96
Mean of variety	3.66	3.84		2.47	1.85	1.22
	Sem (+)	CD (p = 0.01)		Sem (+)	CD (p = 0.01)	
C.D of V (Variety)	0.001	0.004		0.015	0.059	
C.D of R (Isolates)	0.003	0.010		0.034	0.129	
C.D of V*R (Variety *Isolates)	0.004	0.014		0.048	0.018	

The interaction effect of microbial inoculum with varieties DGGV-2 and DGG-96 was found significant in soil dehydrogenase activity at 30, 45 and 60 DAS with a range of 44.23-22.44, 87.19-40.13, 48.51-30.34. The treatment M7 with DGGV-2 recorded significantly highest Soil dehydrogenase activity of 44.23, 87.19, 48.51 at 30, 45 and 60 DAS respectively.

Soil organic carbon

The percentage of soil organic carbon was estimated at 45 and 60 DAS and the results are presented in the Table 2. Inoculation of rhizobial inoculants and consortium for green gram varieties, increased the soil organic carbon (%) of green gram significantly as compared to uninoculated control. Among all observation recorded at 45 and 60 DAS across all different treatments, microbial consortium with M7 inoculum (4.35, 3.00) shown highest percentage soil organic carbon when compared to all treatments followed by M5 inoculum (3.72, 1.96), M6 inoculum (2.29, 1.39) and least soil organic carbon (%) among all treatments was shown by M9 inoculum with percentage of (1.02, 0.19). Among the varieties DGGV-2 with an average of 2.106, 1.50 shown higher soil organic carbon (%) when compared to DGG-96 (2.40, 1.02) at 45 and 60 DAS respectively. The interaction effect of *Rhizobium* and rhizobacteria for

varieties DGGV-2 and DGG-96 was found significant at 45 and 60 DAS. The treatment M7 with DGGV 2 recorded significantly highest percentage of soil organic carbon of 5.43, 4.20 at 45 and 60 DAS respectively.

Soil organic matter

The percentage of soil organic matter was estimated at 45 and 60 DAS and the results are presented in the Table 3. Inoculation of rhizobial inoculants and consortium for green gram varieties, increased the soil organic matter (%) of green gram significantly as compared to uninoculated control. Among all observation recorded at 45 and 60 DAS across all different treatments, microbial consortium with M7 inoculum (7.50, 5.18) shown highest percentage soil organic carbon when compared to all treatments followed by M5 inoculum (6.43, 3.50), M6 inoculum (3.95, 2.57) and least soil organic carbon (%) among all treatments was shown by M9 inoculum with percentage of (1.00, 0.33). Among the varieties DGGV-2 with an average of 3.66, 2.47 shown higher soil organic carbon (%) when compared to DGG-96 (3.84, 1.85) at 45 and 60 DAS respectively. The interaction effect of *Rhizobium* and rhizobacteria for varieties DGGV-2 and DGG-96 was found significant at 45 and 60 DAS. The treatment M7 with DGGV 2 recorded significantly highest percentage of soil organic carbon of

9.37, 7.24 at 45 and 60 DAS respectively.

Discussion

Soil microbial activity can be directly measured using dehydrogenase enzyme activity. Soil dehydrogenase activity indicates the soil biological activity and can be directly correlated with soil health as this enzyme is found in all living microbial cells and express oxidative activity of cells (Casida *et al.*, 1964) [3]. Soil dehydrogenase activity was significantly improved when seeds were treated with consortium when compared to positive controls (NC-92, HR-73, BR-29) and uninoculated control. Compared to all microbial inoculum M7 showed highest Soil dehydrogenase activity at 30, 45 and 60 DAS among the varieties DGGV-2 showed higher values of Soil dehydrogenase activity than DGG-96 and the interaction effect of *Rhizobium* and rhizobacteria was positively correlated in green gram varieties for soil dehydrogenase activity. The interaction effect of DGGV-2 with M7 microbial inoculum treatment shown highest value at 30,45 and 60 DAS when compared to all other treatments. From the observations highest amount of soil dehydrogenase activity was found significantly at 45 DAS could be due to higher microbial load at peak flowering and pod filling stage and a rapid decline at 60DAS indicating the harvest of crop. The results are similar with findings of Sawicka *et al.* (2020) [2] reported that soil enzyme activity was affected by over fertigating fields in Poland and ultimately effecting entire Agro ecosystem. Priyanka *et al.* (2022) [5] in UAS-Dharwad observed increase in soil dehydrogenase activity of green gram plants when seeds are treated with consortium over uninoculated control.

The percentage soil organic carbon and soil organic matter content varied significantly as 45 and 60 DAS and responded positively for microbial inoculation than untreated control by showing higher percentage of soil organic carbon and soil organic matter content than untreated control. The highest soil organic carbon content at 45 and 60 DAS was shown by M7 inoculum among both the varieties and between varieties DGGV-2 has shown higher soil organic carbon percentage than DGG-96. The interaction effect of *Rhizobium* and rhizobacteria with green gram varieties, DGGV-2 with M7 microbial inoculum treatment shown highest value at 45 and 60 DAS. The increase in soil organic carbon and matter might indicate the effective colonization of bacteria that grows in rhizospheric region and effective mobilization of nutrients by beneficial bacteria might have helped the increase in soil organic carbon content. The similar results were recorded by Dkhar *et al.* (2019) [1] who reported soil quality parameters were enhanced by seed inoculation of *Rhizobium* with PSB compared to other treatments. Soil organic carbon and DHA was found significantly at 45 DAS could be due to higher microbial load at peak flowering and pod filling stage and a rapid decline at 60 DAS indicating the harvest of crop.

Conclusion

The present investigation clearly demonstrated that microbial inoculation, particularly in the form of multi-strain consortia, plays a significant role in improving soil health in green gram cultivation. Among the treatments evaluated, the consortium comprising *Rhizobium* strains HR-73 and BR-29 along with rhizobacteria AUDP 203 and AUDP 279 consistently recorded the highest soil

dehydrogenase activity, soil organic carbon, and organic matter content at different growth stages. The peak in dehydrogenase activity and organic carbon observed at 45 days after sowing highlights the strong association between microbial activity and critical crop growth phases such as flowering and pod development.

Between the two green gram varieties, DGGV-2 exhibited superior response to microbial inoculation in terms of soil biological and chemical parameters, indicating varietal differences in rhizosphere interactions and host-microbe compatibility. The significant interaction effects observed between microbial treatments and crop varieties emphasize the importance of selecting appropriate inoculant combinations for specific cultivars.

Overall, the findings confirm that the application of *Rhizobium*-based microbial consortia enhances soil microbial activity and carbon enrichment more effectively than single inoculants or uninoculated controls. Adoption of such bio-inoculant strategies can contribute to sustainable pulse production by improving soil health, reducing dependence on chemical fertilizers, and maintaining long-term soil productivity. The study supports the integration of microbial consortia into green gram cultivation as a viable approach for eco-friendly and sustainable agriculture.

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