



Exploring the Combined Effect of Microbial Consortia and Fertilizers on Growth and Yield of Soybean [*Glycine max* (L.) Merrill.]

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ABSTRACT

Background: Soybean crop require macro and micronutrients, supplemented by fertilizers and manures, but many of them tend to be lost and make them unavailable. Nutrient use efficiency in soybean may be enhanced by deploying certain microorganisms, which have the capacity to solubilize them, increase their availability and facilitate their uptake.

Methods: A three-year field experiment in a randomized block design with three replications was conducted to evaluate the effects of the recommended fertilizer dose, Bio NPK, Bio Zn and microbial consortia individually and in combination on soybean growth and yield.

Result: The positive effects of combined application of fertilizers and microbial consortia containing 75% RDF + Rhizobium + MDSR 14 + 12c led significantly higher plant dry matter production at 60 DAS (18.78 g), root nodules (41.26) and its dry weight (0.49 g), number of pods per plant (58) and seed yield (3104 kg ha⁻¹) over the control and was at par with 100% RDF. An increase in seed yield with microbial consortia coupled with inorganic fertilizers was 37.41%, 3.33% and 11.25% over the control, 100% RDF and 75% RDF, respectively. Economically, the treatment T7 containing inorganic fertilizers and microbial consortia was promising in terms of maximum net returns (Rs. 80,346/- ha⁻¹) and benefit-cost ratio (2.99). Soybean crop supplemented with 75% RDF and seed inoculation with rhizobium + MDSR 14 + 12c microbial consortia (T7) was observed to save 25% of the recommended dose of fertilizer and gave at par yield with 100% RDF.

Key words: Nutrient availability, Phosphate solubilization, Plant growth-promoting bacteria, Plant nutrition, Soil fertility.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill.] is an important legume and oilseed crop. It is known as a miracle bean due to its wide utility as food, fodder and domestic and commercial uses. Soybean is a highly adaptive crop and grows in diverse climatic conditions with good yield potential under optimum cultivation practices. It emerged as a nutritionally important commodity due to its high-quality protein and edible oil. In addition, soybean, being a legume crop, fixes atmospheric nitrogen (N₂) in the soil (Jordan, 1982). Hence, it is a soil fertility restorer crop suitable for intercropping, crop rotation and sequence cropping (Singh *et al.*, 2018). Due to its promising returns within a short period, soybean is becoming increasingly popular among farmers and is being cultivated across diverse agro-ecologies (NAAS, 2017).

India ranks fifth in soybean production on the global front. The area under the soybean crop in India is increasing steadily and presently, it is cultivated in an area of 11.8 M ha with a production of 11.87 MT and productivity of 1 T ha⁻¹ (SOPA, 2023). However, compared to the world's (3.1 t ha⁻¹) average productivity, it is less (1.10 t ha⁻¹). There is a need to bridge this gap to meet the increasing food demand of the growing population. Madhya Pradesh (43.90%) and Maharashtra (38.50%) are the leading soybean-producing states of India (SOPA, 2023). However, with an increased

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area under cultivation and the development of suitable high-yielding soybean varieties, production has increased, but productivity is still on the lower side. Hence, ardent attention needs to be given to various factors associated with low productivity like crop management, balanced nutrition, irrigation, post-harvest management, etc., which help in sustainable soybean cultivation.

Soybean crop requires macro (N, P and K) and micro (Ca, Mg, Mn, S, B, Fe, Zn, Mo, etc.) nutrients for growth, development and metabolic activities associated with

photosynthesis and completion of the life cycle. To supply these nutrients, organic and inorganic fertilizers are applied as seed treatment, basal dose and foliar application following the recommended dose as specified. Moreover, many of them tend to lose due to leaching, adsorption and fixation with other elements in soil, consequently becoming unavailable to the plants, influencing their use efficiency and uptake by the plant (Ibrahim *et al.*, 2022). This results in the wastage of resources and financial losses and causes harmful effects on the environment. An application of organic fertilizers alone is not feasible as they are slow nutrient-releasing and require in high quantities. For efficient utilization of the applied nutrients through different sources like bacterial strains in the form of microbial consortia or plant growth-promoting bacteria (PGPB) for seed inoculation or soil application can be useful by producing certain enzymes that help in transforming insoluble nutrients in the available form (Mahdi *et al.*, 2012; Virk *et al.*, 2022). Hence, the present investigation was undertaken to deploy certain microbial bioformulations capable of nutrient solubilization, increasing its availability and facilitating its uptake by soybean plants and their effect on their growth and yield.

MATERIALS AND METHODS

A field experiment was carried out during the Kharif season for three years, from 2021 to 2023, at the experimental farm of Agharkar Research Institute, Pune (MS), India. The experimental site is situated at 18°14' N latitude, 75°21' E longitude and at an altitude of 548.6 MSL. The soil of the experimental site was well-drained and the vertisol had a slightly alkaline pH (7-7.4). The study area is characterized by a sub-tropical climate with an annual average rainfall of 500-750 mm. A field experiment was laid out in completely randomized block design (RBD) with three replications, containing six nutrient management practices comprising a supply of recommended dose of fertilizer (RDF) through inorganic fertilizers, biological NPK and microbial consortia and control, as T1: Control (without supplementing any nutrition), T2: 100% RDF, T3: 75% RDF, T4: 75% RDF + Bio Zn, T5: 75% RDF + Bio NPK, T6: 75% RDF + Bio Zn + Bio NPK and T7: 75% RDF + Rhizobium + MDSR 14 +12c (12c: *Burkholderia arboris*- High P solubilizing bacteria). The popular soybean variety 'DSb 21', recommended for this region was sown after the onset of monsoon during the fourth week of June at a seed rate of 62.5 kg ha⁻¹ on a flatbed with a distance of 45 cm × 5 cm. The gross plot size was 3.6 m × 6 m and the net plot size was 2.7 m × 5 m. All the treatments were assigned as per the experimental layout, following the standard protocol of fertilizer, microbial consortia and biofertilizer application. The crop was grown, maintained healthy and harvested manually after maturity. The recommended dose of fertilizer (20:80:20 NPK kg ha⁻¹) was supplied through di-ammonium phosphate (18:46:00 N: P: K), single super phosphate (00:16:00 N: P: K) and muriate of potash (00:00:60 N: P: K) as basal dose during

sowing. Before sowing 'Bio Zn', a liquid formulation containing Zinc solubilizing bacteria- *Bacillus endophyticus*, 'Bio NPK' having *Azotobacter chroococcum* for N, *Paenibacillus tylopili* for P and *Bacillus decolorationis* for K solubilizing bacterial formulation commercialized by ICAR NABAIM, Kushmaur, U.P. (India), 'MDSR 14' (*Bacillus aryabhatai*) a bacterial strain capable of solubilizing Zinc (Identified by ICAR NSRI Indore, M.P. (India) and '12c' (*Burkholderia arboris*) which is high P solubilizing bacteria isolated from soybean nodules at ICAR NSRI Indore, M.P. (India) were inoculated to the seeds @ 250 ml ha⁻¹ in respective treatments prior to sowing.

The data on morphological and other traits of the experimental crop were recorded to assess the response of soybean to microbial consortia and inorganic fertilizer. Observations on growth and yield attributes were recorded on the randomly selected five plants from each treatment. The crop growth rate (CGR) and relative growth rate (RGR) based on the dry matter content of plants were determined using a standard formula given by Watson (1947) and Williams (1946). The data on root nodules and their dry weight were recorded at the R2 and R5 stages of growth. Soil samples from all treatment plots were collected before sowing and after harvest of the crop in every season and analyzed for available N, P and K as per the modified Kjeldahl method, calorimetrically by tri-acid digestion and by flame photometer, respectively, as described by Jackson (1973). Similarly, the seed and stover samples were analyzed for N, P and K content to compute their uptake using the standard formula described by Deka *et al.* (2019). Based on the soil nutrient availability status, nutrients applied and nutrient uptake by plants, the NPK balance was computed. The economics of the treatments were determined in terms of gross returns, net returns and benefit-cost ratio, considering the prevailing market price of soybean grains. An additional cost of cultivation and net returns over the control were estimated to determine the incremental benefit: cost ratio. The collected data were subjected to analysis of variance (ANOVA) using standard variance techniques suggested by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Effect on soybean growth attributes

Results of the present study revealed that soybean crop supplied with nutrition through microbial consortia and fertilizers had shown a significant increase in plant dry matter content at the later growth stage of the crop (Table 1). Plant dry matter at 60 DAS was significantly higher under the treatment T7 (18.78 g) over the treatment T1 (13.88 g) and T3 (16.11 g) and it was closely followed by T5 (18.65 g), T6 (18.65 g), T4 (17.24 g) and T2 (17.11 g). The positive effect on plant dry matter was observed in the treatments where inorganic fertilizers (75% RDF), rhizobium biofertilizer, zinc solubilizing MDSR 14 and 12c-*Burkholderia arboris* phosphate solubilizing bacteria supplied to the soybean crop. This might have helped to improve the plant

Table 1: Soybean crop growth response to the fertilizers and microbial formulation (Pooled means).

Treatments	Plant height (cm)	Dry matter plant ⁻¹				CGR				RGR				Root nodulation			
		30		45		60		30-45		45-60		30-45		45-60		At R2 stage	
		DAS		DAS		DAS		DAS		DAS		DAS		DAS		Nodule dry wt. (g)	
		plant ⁻¹		plant ⁻¹		plant ⁻¹		plant ⁻¹		plant ⁻¹		plant ⁻¹		plant ⁻¹		Nodule dry wt. (g)	
T1: Control	52.42	2.22	7.36	13.88	0.342	0.486	0.0203	0.0345	0.0203	0.0203	0.0203	0.0345	0.0203	0.0203	0.0203	0.210	0.320
T2: 100% RDF	53.07	2.58	8.32	17.11	0.407	0.597	0.0216	0.0339	0.0216	0.0216	0.0216	0.0339	0.0216	0.0216	0.0216	0.260	0.360
T3: 75% RDF	52.04	2.76	8.34	16.11	0.372	0.528	0.0197	0.0320	0.0197	0.0197	0.0197	0.0320	0.0197	0.0197	0.0197	0.250	0.360
T4: 75% RDF + Bio Zn	55.27	2.45	7.31	17.24	0.324	0.66	0.0248	0.0316	0.0248	0.0248	0.0248	0.0316	0.0248	0.0248	0.0248	0.300	0.450
T5: 75% RDF + Bio NPK	53	2.5	7.23	18.65	0.316	0.679	0.0256	0.0309	0.0256	0.0256	0.0256	0.0309	0.0256	0.0256	0.0256	0.250	0.430
T6: 75% RDF + Bio Zn + Bio NPK	51.13	2.32	8.26	18.65	0.396	0.605	0.0216	0.0359	0.0216	0.0216	0.0216	0.0359	0.0216	0.0216	0.0216	0.310	0.380
T7: 75% RDF + Rhizobium + MDSR 14 + 12c	53.93	2.42	8.78	18.78	0.424	0.605	0.0236	0.0377	0.0236	0.0236	0.0236	0.0377	0.0236	0.0236	0.0236	0.320	0.490
SEm (±)	2.40	0.20	0.60	0.75	0.040	0.068	0.003	0.002	0.003	0.003	0.003	0.002	0.003	0.003	0.003	0.033	0.018
CD (P<0.05)	NS	NS	NS	2.13	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.050

12c=*Burkholderia arboris*-High P solubilizing, CGR: Crop growth rate (g m⁻² day⁻¹), RGR: Relative growth rate (g g⁻¹ day⁻¹).

metabolism, contributing to cell division and elongation, leading to enhanced plant dry matter content during the later growth stage of soybean due to the combined positive effect of inorganic fertilizer and biofertilizers (Timofeeva *et al.*, 2022). The treatment 100% (T2) and 75% (T3) RDF showed a significant increase in plant dry matter at 60 DAS, emphasising the importance of conventional inorganic fertilizers and biofertilizers for soybean crop (Samad *et al.*, 2024). The differences for the plant height, branches per plant, dry matter at 30 and 45 DAS, crop growth rate and relative growth rate were observed non significantly different under the treatments studied.

Effect on root nodules and their dry weight

The data on the effect of the fertilizer and bioformulation consortia on the root nodulation are presented in Table 1. Root nodulation and its dry weight at the R1 stage were not statistically significant. However, the fertilizer and bioformulation treatments significantly improved the average number of root nodules per plant and their dry weight at the R5 stage. A significant maximum average number of nodules and their dry weight were recorded in treatment T7 (41.26 nos. and 0.490 g), while the least were in treatment T1 (21.27 nos. and 0.320 g) and T2 (30.38 nos. and 0.36 g). Application of the different inoculants having the capacity to fix the atmospheric nitrogen in the soil, as well as zinc and phosphate solubilization ability in soil, along with inorganic fertilizers, might have resulted in the optimum availability of nitrogen and phosphorus in the soil, leading to improved nodulation and their dry weight (Javed *et al.*, 2023; Lingaraju *et al.*, 2016). Similar results of the increase in nodule number and their dry weight due to inoculation of *bradyrhizobium japonicum* along with the N and P fertilizers have been reported by Wei *et al.* (2023) and Zveushe *et al.* (2023).

Effect on yield and its attributes

The number of pods per plant and seed yield was significantly different due to the various treatments of fertilizer and microbial consortia inoculation (Table 2). The number of pods per plant and seed yield was significantly higher in T7 (58 nos. and 3104 kg ha⁻¹) containing fertilizer and microbial consortia over the control (39 nos. and 2259 kg ha⁻¹). The seed yield obtained under the 100% RDF (T2) was at par with the treatments 75% RDF (T3) and 75% RDF in combination with Bio Zn and Bio NPK (T4, T5 and T6). The results reveal that the fertilizers and microbial consortia improved seed yield by 37.41% over control (T1) and 3.33% over 100% RDF (T2). Improved seed yield and number of pods per plant due to the combined effect of fertilizer and microbial consortia inoculation might have been attributed to an increased microbial abundance and their activity led to enhanced soil fertility, improved nutrient availability, their uptake by plants and there by contributing to yield (Miljakovic *et al.*, 2024; Argaw, 2012). The results suggest that the reduction in the recommended dose of fertilizers to the tune of 25% coupled with an application of the microbial consortia would be able to bring out a

Table 2: Effect of fertilizers and microbial consortia on yield and its attributes of soybean (Pooled means).

Treatments	No. of pods plant ⁻¹	Biological yield (kg ha ⁻¹)	Straw yield (kg ha ⁻¹)	Harvest index (%)	Seed index (g)	Yield (kg ha ⁻¹)	% increase over control (T1)
T1: Control	39	5308	3049	43.41	11.98	2259	-
T2: 100% RDF	51	6110	3123	49.42	12.27	3004	32.98
T3: 75% RDF	43	6023	3216	46.98	12.32	2790	23.51
T4: 75% RDF + Bio Zn	46	5959	3042	49.10	12.00	2917	29.13
T5: 75% RDF + Bio NPK	54	6080	3106	49.71	12.30	2974	31.65
T6: 75% RDF + Bio Zn + Bio NPK	54	6041	2996	51.11	11.90	3046	34.84
T7: 75% RDF + Rhizobium + MDSR 14 + 12c	58	6231	3094	51.13	12.52	3104	37.41
SEM (±)	1.90	393.1	275	1.88	0.57	164	
CD (P≤0.05)	5.50	NS	NS	NS	NS	466	

Table 3: Economics of the soybean crop grown with fertilizers and bioformulations (Pooled means).

Treatments	Yield (kg ha ⁻¹)	Cost of cultivation (Rs. ha ⁻¹)	Gross returns (Rs. ha ⁻¹)	Net returns (Rs. ha ⁻¹)	B: C ratio	Additional cost of cultivation over control (Rs. ha ⁻¹)	Additional net returns over control (Rs. ha ⁻¹)	Incremental benefit: cost ratio
T1: Control	2259	33642	90446	56804	2.69	-	-	-
T2: 100% RDF	3004	43420	119576	76156	2.75	9778	19352	1.98
T3: 75% RDF	2790	41064	111126	70062	2.7	7422	13258	1.79
T4: 75% RDF + Bio Zn	2917	41414	116116	71702	2.80	7772	14898	1.92
T5: 75% RDF + Bio NPK	2974	41414	118407	76993	2.85	7772	20189	2.60
T6: 75% RDF + Bio Zn + Bio NPK	3046	41664	121312	79647	2.91	8022	22843	2.85
T7: 75% RDF + Rhizobium + MDSR 14 + 12c	3104	41314	123661	80346	2.99	7672	23542	3.07
SEM (±)	164	375	5704	5372	0.12			
CD (P≤0.05)	466	1064	16171	15231	NS			

Table 4: Soil nutrient status and nutrients added every year to the soybean crop grown (Pooled means).

Treatments	Initial available soil NPK (kg ha ⁻¹) [a]			Nutritional content after harvest (kg ha ⁻¹) [b]			Nutrients added (kg ha ⁻¹ every year) [c]			Nutrient uptake (kg ha ⁻¹ by the whole system) [d]			NPK (kg ha ⁻¹) balance [e]: (a + c) – (b – d)		
	N	P	K	N	P	K	N	P	K	N	P	K	N	P	K
T1: Control	190.13	38.03	375.17	164.92	34.73	310.41	0.00	0.00	0.00	174.17	13.54	71.20	199.38	16.84	135.96
T2: 100% RDF	178.96	40.46	326.77	183.47	31.73	271.03	20.00	80.00	30.00	205.81	15.99	76.35	221.29	104.73	162.09
T3: 75% RDF	178.86	41.46	357.55	153.41	37.35	308.00	15.00	60.00	22.50	210.53	15.06	78.81	250.98	79.17	150.86
T4: 75% RDF + Bio Zn	163.12	33.80	375.75	154.57	44.22	368.46	15.00	60.00	22.50	208.74	15.99	75.94	232.29	65.58	105.74
T5: 75% RDF + Bio NPK	175.20	36.49	299.62	158.08	38.52	296.82	15.00	60.00	22.50	216.13	18.45	85.35	248.25	76.42	110.65
T6: 75% RDF + Bio Zn + Bio NPK	168.28	31.37	319.67	173.83	44.41	330.62	15.00	60.00	22.50	226.06	18.04	81.24	235.51	65.01	92.79
T7: 75% RDF + Rhizobium + MDSR 14 + 12c	200.77	32.26	290.16	210.72	36.85	338.88	15.00	60.00	22.50	242.53	24.86	94.78	247.58	80.26	68.57

significant increase in the seed yield of soybean and was at par with the 100% RDF, these results are in conformity with the findings of Somanagouda *et al.* (2024).

Economics of the study

Economic analysis of the different treatments (Table 3) revealed that an application of fertilizer along with the microbial consortia incurred a higher cost of cultivation over the control, while it was less compared to 100% RDF. The maximum cost of cultivation was incurred in treatment consisting of 100% RDF (Rs. 43,420/- ha⁻¹), while the least was in the control (Rs. 33,642/- ha⁻¹). The gross returns (Rs. 1,23,661/- ha⁻¹) and net returns (Rs. 80,346/- ha⁻¹) were significantly higher in the treatment consisting of 75% RDF + Rhizobium + MDSR 14 + 12c over the control (Rs. 90,446/- ha⁻¹ and Rs. 56,804/- ha⁻¹, respectively) and was closely followed by the rest of the treatments. Higher gross and net returns under treatment T7 attributed to an increase in yield due to a positive response to the bioformulation of microbial consortia, saving the cost on fertilizers, resulting in a higher benefit-cost ratio and additional net returns of Rs. 4,190/- ha⁻¹ (21.65%) over the 100% RDF. An incremental benefit-cost ratio (3.07:1) determined based on an additional cost of cultivation and net returns over the control was high with the treatment 75% RDF + Rhizobium + MDSR 14 + 12c and least with the treatment 75% RDF.

Soil nutrient status, nutrient uptake and balance sheet

The results of soil and plant analysis (Table 4) showed that the total uptake of N varied from 242.53 kg ha⁻¹ to 174.17 kg ha⁻¹, that of P from 24.86 kg ha⁻¹ to 13.54 kg ha⁻¹ and K from 94.78 kg ha⁻¹ to 71.20 kg ha⁻¹. Among the treatments, T7: 75% RDF + Rhizobium + MDSR 14 + 12c recorded maximum uptake of nitrogen, phosphorus and potassium over the control, which was followed by treatment T6 for N and T5 for P and K. Improved N, P and K uptake might have resulted due to the higher availability of these nutrients from soil, fertilizers and microbial consortia. These results corroborate the findings of Jaga and Sharma (2015) and support that the combined application of fertilisers and biofertilizers helps in augmenting the yield. However, the N, P and K uptake under the treatment of 100% RDF was less compared to the treatments containing microbial consortia. This might be due to the treatment containing bioformulation, which recorded higher N, P, K and Zinc availability to plants and dry matter accumulation led to higher nutrient uptake compared to RDF only (Kumawat *et al.*, 2021; Yaduwanshi *et al.*, 2018). Higher nitrogen balance was observed in the treatment containing a combination of fertilizer and bioformulation with 75% RDF followed by 75% and 100% RDF. The lowest N balance was observed in the treatment control (T1). In the case of P and K, the balance was higher in 100% RDF and 75% RDF treatments and the lowest was under the treatment control (T1).

CONCLUSION

The study revealed that the use of microbial consortia, along with fertilizers, maintains soil fertility, supplies essential nutrients to soybean, improves its growth and yield sustainably and eco-friendly and helps in reducing fertilizer costs.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the committee of experimental animal care and handling techniques were approved by the university of animal care committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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