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Effect of Microbial Consortia on Soil Health, Nutrient Uptake and Yield of Soybean Grown in a Vertisol of Central India

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Abstract

A field experiment was carried out in the research farm of the Jawaharlal Nehru Krishi Vishwa Vidhyalaya, Jabalpur, Madhya Pradesh, India during *Kharif* season of 2021-22. The experiment was performed with nine treatments comprised by *Rhizobium, Pseudomonas,* EM and their consortia with FUI and UFUI (absolute control) in randomized block design. The soybean crop was grown in the field along with recommended package of practices. The result showed that the yield of soybean was 43% higher (2351 kg ha^{-1}) than control FUI and better uptake of N (145 kg ha^{-1} by seed and 125.6 kg ha^{-1} by straw), P (7.9 kg ha^{-1} by seed and 14.9 kg ha^{-1} by straw) and K (42.3 kg ha^{-1} by seed and 65.0 kg ha^{-1} by straw) by soybean were found due to inoculation of microbial consortia of *Rhizobium, Pseudomonas* and EM culture. The Physico-chemical properties (pH, EC and OC), availability of nutrients (NPK with vales of 288, 29 and 335 kg ha^{-1} respectively), enzymatic activities (Acid phosphatase, alkaline phosphatase and dehydrogenase activities with values of 42.2, 69. 8 µg PNG h^{-1} g^{-1} of soil and 12.9 µg TPF hr^{-1} g^{-1} of soil, respectively) and microbial population (*Rhizobium* by 35.1×10^{6}, total bacteria by 39.3×10^{7}, fungi by 41.9×10^{4} and actinomycetes by 38.7×10^{3} cfu g^{-1} soil) in soil also improved with the same treatment as compared to FUI. It is strongly recommended that the inoculation of microbial consortia improved the soil health and soybean production too instead of single inoculation by modulating microbial population which improves the soil health by nutrient fixation, solubilization and mobilization and plant growth promotion.

Keywords: Nutrient assimilation, *Pseudomonas*, EM, DHA, vertisol

1. Introduction

Soybean, extensively cultivated in India, particularly in Madhya Pradesh, thrives across diverse agro-climatic conditions and commands a high market value. It stands out for its nutritional richness, containing 42–43% protein, 18–20% edible oil with essential fatty acids, lecithin, and vitamins A and D, coupled with significant carbohydrate (30%) and fiber (4%) content. In the Indian context, soybean cultivation spans 11.34 mha, with an expected yield of 11.99 mt in the 2021–2022. Madhya Pradesh leads both in terms of cultivated area (5.51 mha) and production (5.56 mt).

However, concerns arise from conventional agricultural practices heavily reliant on inorganic inputs, impacting the nutritional quality of produce and the well-being of farmers and consumers (Kumar et al., 2021). Addressing these problems, microbial inoculants have emerged as natural compounds with the potential to manage pests, improve soil and crop quality, and positively influence human health. The soybean rhizosphere, hosting a myriad of soil microorganisms, plays a pivotal role in shaping soil biology and influencing plant growth from the seedling stages. Inoculation with microbial strains like *Rhizobium* and phosphate-solubilizing microorganisms has proven effective in enhancing crop productivity and soil fertility (Sahu et al., 2023a). While rhizobia, renowned for their ability to colonize the rhizosphere and form nodules in plant roots, serve as traditional inoculants, recent research suggests that combining *Rhizobium* with other beneficial microorganisms such as *Pseudomonas* and EM in consortia could yield enhanced benefits. These include improved nutrient supply through solubilization, anti-phytopathogenic properties, induced phytoresistance, and phytostimulation.

Under specific conditions, certain rhizobacteria significantly impact plant growth and development (Sahur et al., 2018). Furthermore, microbial consortia contribute to sustainable agriculture by mitigating the environmental impact of agrochemicals is designed with the objective of optimizing soybean harvests under prevailing climatic and soil conditions.

2. Materials and Methods

The experiment was conducted at the research fields of Department of Soil Science and Agricultural Chemistry, JNKVV, Jabalpur (M.P.). The site is situated from 23°13" North latitude to 79°57" East longitude and 393 meter high from the mean sea level. It is located in the northern section of Madhya Pradesh, India. The tropic of cancer runs through the middle of the district. In December, the average maximum and minimum temperatures range from 36.6.1 to 44.4°C in June and 8 to 9°C in January, respectively. The average relative humidity was 38% in the nights and 82% in the morning. The average annual rainfall was 1160 mm, with the majority falling between July and September due to the south-west monsoon. However, there was a probability of precipitation during the colder months (December and January). The soils of the experimental field belong to Kheri series and taxonomically come under the order Vertisol of fine montmorillonitic hyperthermic family of *Typic Haplusterts*. The experiment was laid out under RBD design along with three replication and nine treatments comprises : T_1: *Rhizobium*, T_2: *Pseudomonas*, T_3: EM, T_4: Rhizo+Pseudo, T_5: Rhizo+EM, T_6: Pseudo+EM, T_7: Rhizo+Pseudo+EM, T_8: FUI, T_9: UFUI where EM culture is a consortium of *Rhodopseudomonas palustris, Lactobacillus lactis, Saccharomyces cerevicea, Streptomyces badius* and *Aspergillus niger*.

2.1. Soil sampling and analysis

The initial soil samples (0–15 cm depth) were drawn from 10 to different places throughout the study field and combined together to generate a representative composite sample. While plot wise soil sampling was done after the harvest of crop. The soil pH of the samples was determined in 1:2.5 substrate water suspension by using digital pH meter (Jackson, 1973). The electrical conductivity was determined by conductivity meter (Jackson, 1973). Soil organic-carbon was estimated following the method described by Walkley and Black (Walkley and Black, 1934). The available N, P and K in soil were determined by alkaline permanganate method (Subbiah and Asija, 1956), ascorbic acid method (Olsen et al., 1954) and neutral normal ammonium acetate method by using flame photometer (Hanway and Heidel, 1952) respectively.

Rhizospheric soil samples were utilized without being grinded sieved and altered. The collected samples were kept in low density polythene bags in the refrigerator at 4°C, but microbiologically analyzed as soon as possible. Serial dilution was performed by suspending 10 g of soil sample in 90 ml sterilized water in flasks and dilutions were made up to 10-8. Each dilution was plated in triplicate. The composition of growth medium for respective microorganisms *viz.* YEMA, Sodium Albuminate Agar, Rose Bengal streptomycin Agar (RBSA) and Starch Caseinate Agar medium for growth of *Rhizobium*, total bacteria, fungi and actinomycetes respectively, were used. Soil dehydrogenase is a direct measurement of soil microbial activity indicating the microbial mechanisms going on in the soil. It was monitored in the soil samples collected from experimental site. DHA method is developed by Casida et al. (1964). The method suggested by Tabatabai and Bremner (1969) was used to analyse phosphatase activity.

The soybean crop (Var. JS-2098) was seeded on 11th July, 2021 and harvested on 17th November, 2021 by adopting all the recommended package of practices. After threshing individually seed and stover samples were weighed (kg ha^{-1}) and data were recorded plot-wise. Samples were collected from each plot for further NPK analyses in seed and stover. The seed and stover of soybean was taken to determine nutritional consistence. For the determination of nitrogen content in soybean crop micro Kjeldahl method was used (Anonymous, 1995), for phosphorus content of the plant extract was determined using Vanado-Molybdo phosphoric yellow colour method (Bhargava and Raghupathi, 1984) and potassium was determined with the help of flame photometer (Bhargava and Raghupathi, 1984). On the basis on nutrient content the nutrient uptake of soybean was calculated in kg ha^{-1}.

2.2. Statistical analysis

All the recorded data were statistically analyzed by using a randomized block design to determine the statistical significance of variance among different treatments means as influenced by the application of microbial treatment on various soybean properties (Gomez and Gomez (1984).

3. Results and Discussion

3.1. Yield

The findings of the study indicate that the introduction of microbial inoculation, particularly the combination of Rhizo+Pseudo+EM, has a significant positive impact on soybean grain yield, leading to a substantial increase of 43% over the control group (FUI), resulting in a yield of 2351 kg ha^{-1} (Table 1). The observed improvement in soybean yield is attributed to enhanced nodulation, nitrogen fixation (N_2), and overall crop growth facilitated by the microbial inoculation. Furthermore, co-inoculation with specific strains also demonstrated promising outcomes in enhancing soybean productivity. It is noteworthy that the references to Sahu et al. (2023a) indicate that the current study aligns with existing research findings.

3.2. Nutrients uptake

The outcome of the study reported with regard to nutrient uptake by soybean in Table 1. The combination treatment of

Table 1: Impact of microbial consortia on yield and nutrient uptake by soybean

Treatment		Yield (kg ha^{-1})		Nutrient uptake (kg ha^{-1})					
				N		P		K	
		Seed	Stover	Seed	Stover	Seed	Stover	Seed	Stover
T_1	Rhizobium (Rhizo)	1784	3680	108.7	89.2	6.2	8.9	27.8	52.1
T_2	Pseudomonas (Pseudo)	1839	3915	113.2	82	6.0	9.6	29.6	53.1
T_3	EM culture	1949	4273	124.4	94.2	6.3	11.0	31.3	57.7
T_4	Rhizo+Pseudo	1981	4255	128.9	96.6	6.4	11.0	33.8	58.2
T_5	Rhizo+EM	2040	4165	133.5	107.3	6.7	11.5	35.4	60.3
T_6	Pseudo+EM	2108	4371	132.7	109.1	7.1	11.9	39.0	62.6
T_7	Rhizo+Pseudo+EM	2351	5387	145.0	125.6	7.9	14.9	42.3	65.0
T_8	FUI	1642	3348	98.5	83.6	5.7	7.7	25.0	55.0
T_9	UFUI	1552	3070	90.1	76.4	5.0	6.9	23.7	51.2
Mean		1916	4051	121	91.7	6.4	10.4	32	59.4
SEm±		99.7	317.4	8.5	7.05	0.28	0.99	1.81	5.42
LSD (p=0.05)		302.4	954.6	22.7	19.7	0.85	2.58	4.88	17.1

Rhizo+Pseudo+EM exhibited the highest uptake of 156.0 kg N ha^{-1} and 145.6 kg N ha^{-1} by the seeds and stover, respectively which were significantly over uninoculated (FUI) control. These findings align with Nimnoi et al. (2014), who emphasized the positive effects of endophytic actinomycetes as co-inoculants with *Bradyrhizobium japonicum.* Additionally, Kumar et al. (2021) supported the study which reinforcing the significance of microbial inoculation in improving nutrient uptake by soybean plants. The maximum phosphorus uptake of 9.3 kg P ha^{-1} and 14.9 kg P ha^{-1} by seeds and stover, respectively was recorded in the Rhizo+Pseudo+EM treatment which was significantly superior to FUI and followed by PGPR+EM and Rhizo+EM. The stimulatory effects of Pseudomonas and Bacillus, were identified as contributing factors to the observed enhancements in phosphorus uptake of cotton by Sahu et al. (2023a).

However, the highest uptake of K by seed and stover (42.3 and 82.0 kg K ha^{-1}) was found with the application of microbial combination of Rhizo+PGPR+EM. This outcome is likely attributed to the potential improvement in K nutrition facilitated by the inoculation of PGPR as a biofertilizer and secretion of organic acids mediated by soil microorganisms (Sahu et al., 2023a).

3.3. *Soil fertility status*

The findings of the experiment related to pH, electrical conductivity (EC) and soil organic carbon (SOC) presented in Table 2. Irrespective of the treatment there are no significant differences were noticed in pH and EC. Almost neutral values of pH and safe range of EC were recorded among all the treatments. The significantly (p<0.05) higher value of SOC was reported 7.0 g kg^{-1} of soil by the application of microbial consortium Rhizo+Pseudo+EM over fertilized uninoculated control (6.1 g kg^{-1} of soil). This was followed by the treatments Pseudo+EM and Rhizo+EM associated with the values of 6.8 and 6.7 g kg^{-1} of soil respectively. Overall, it was noticed the microbial consortia of *Rhizobium*, *Pseudomonas*, EM culture responded better performances over the single inoculation. This was probably due to the rapid decomposition of fresh or immature organic material and the intensive polymerization process (humification) of organic matter as influenced by the microbial consortia. These results were in accordance to Tiwari et al. (2023).

The utilization of the microbial combination Rhizo+Pseudo+EM resulted in a noteworthy increase in the available N, P and K content of 288.4 kg ha^{-1}, 29.2 kg ha^{-1} and 335.1 kg ha^{-1}, compared to over FUI 199.4 kg ha^{-1}, 18.7 kg ha^{-1} and 245.3 kg ha^{-1}, respectively (Table 2). This availability of nitrogen can be attributed to the increased proliferation of soil microbes, which play a crucial role in converting organically bound nitrogen into an inorganic form, as documented by Katkar et al. (2006). Furthermore, the production of growth-promoting substances and the robust colonization ability of rhizobacteria, *Pseudomonas,* contribute to enhanced nitrogen fixation in soybeans when co-inoculated with *B. japonicum* (Yaduwanshi et al., 2021 and Tekam et al., 2023). Similar findings were reported by Sarkar et al. (2002), who observed that actinomycetes, in conjunction with PGPR and Arthrobacter, demonstrated the capability to solubilize tricalcium phosphate, with rock phosphate being less soluble than calcium phosphate. Certain bacteria, fungi, and actinomycetes are known to solubilize potassic minerals into a soluble form through enzymatic oxidation-reduction reactions, as well as the formation of chelates and complexes with proteins, amino acids, organic acids, etc.

Table 2: Impact of microbial consortia on basic properties of soil

Treatment		pH	EC (dS m^{-1})	SOC (g kg^{-1})	Available nutrients (kg ha^{-1})		
					N	P	K
T_1	*Rhizobium* (Rhizo)	6.6	0.27	6.5	218.3	20.3	254.6
T_2	*Pseudomonas* (Pseudo)	6.2	0.28	6.2	226.0	18.7	259.4
T_3	EM culture	6.3	0.29	6.5	237.3	20.5	265.7
T_4	Rhizo+Pseudo	6.4	0.25	6.6	245.4	22.2	277.8
T_5	Rhizo+EM	6.7	0.29	6.7	253.0	25.5	296.4
T_6	Pseudo+EM	7.1	0.28	6.8	260.2	27.6	317.0
T_7	Rhizo+Pseudo+EM	7.0	0.26	7.0	288.4	29.2	335.1
T_8	FUI	6.3	0.25	6.1	199.4	18.7	245.3
T_9	UFUI	6.4	0.25	6.0	191.8	17.5	240.6
Mean		6.5	0.27	6.5	235.5	22.2	276.3
SEm±		0.23	0.012	0.15	19.05	2.7	23.7
LSD (p=0.05)		0.66	0.040	0.460	57.12	6.3	67.2

3.4. Soil enzymatic properties

The phosphatase (acid and alkaline) enzymes convert organic phosphorus molecules into inorganic accessible phosphorus for plant nourishment. While dehydrogenases are one of the most important enzymes and used as an indicator of soil microbial activity. The maximum activity of acid, alkaline phosphatase and Dehydrogenases activities (Table 3) in rhizospheric soil of soybean were monitored highest 42.2, 69.8 µg PNG h^{-1} g^{-1} soil and 15.9 µg TPF h^{-1} g^{-1} of soil by the inoculation of microbial consortia Rhizo+Pseudo+EM followed by Pseudo+EM over control (FUI). This result of investigation is in corroboration with the findings of Rana et al. (2015), Thakur et al. (2022) and Jabborova et al. (2020) confined 52% and 103% increase in acid and alkaline phosphatase activity in soil respectively over the uninoculated control in oilseed rape when co-inoculated with *Pseudomonas and Enterobacter* strains. However the enhanced activity of the dehydrogenase may be due to a change in the composition of the root exudates in the soybean rhizophore. Root exudates

Table 3: Impact of microbial consortia on enzymatic and microbial status of soil

Treatment		Enzymatic activities		
		Phosphatase (µg PNG h^{-1} g^{-1} soil)		DHA (µg TPF hr^{-1} g^{-1})
		Acid	Alkaline	
T_1	*Rhizobium* (Rhizo)	34.2	47.4	8.3
T_2	*Pseudomonas* (Pseudo)	35.5	54.1	8.5
T_3	EM culture	36.6	60.0	9.7
T_4	Rhizo+Pseudo	37.6	62.9	10.9
T_5	Rhizo+EM	38.2	64.6	8.4
T_6	Pseudo+EM	39.4	71.3	11.4
T_7	Rhizo+Pseudo+EM	42.2	69.8	12.9
T_8	FUI	33.1	48.0	8.4
T_9	UFUI	29.7	42.4	6.0
Mean		36.3	57.8	9.7
SEm±		2.29	2.11	0.54
LSD (p=0.05)		6.85	6.31	1.60

Table 4: Impact of microbial consortia on enzymatic and microbial status of soil

Treatment		Enzymatic activities			
		Rhizobium	Bacterial	Fungi	Actinomycetes
		$x10^6$	$x10^7$	$x10^4$	$x10^3$
T_1	*Rhizobium* (Rhizo)	19.7	21.3	26.1	18.1
T_2	*Pseudomonas* (Pseudo)	22.6	25.7	28.3	27.3
T_3	EM culture	24.8	27.2	33.5	29.9
T_4	Rhizo+Pseudo	24.9	27.3	39.7	27.1
T_5	Rhizo+EM	26.2	29.3	38.2	27.4
T_6	Pseudo+EM	28.1	32.1	40.1	32.4
T_7	Rhizo+Pseudo+EM	35.1	39.3	41.9	38.7
T_8	FUI	22.4	21.1	30.3	25.3
T_9	UFUI	10.9	19.3	16.7	15.7
Mean		24.1	26.7	33.4	27.2
SEm±		7.3	6.2	1.01	5.24
LSD (p=0.05)		4.1	5	3.21	7.11

have a significant impact on rhizosphere microorganisms, both qualitatively and quantitatively (Gorde et al., 2022).

3.5. Microbial health

The microbial consortium Rhizo+Pseudo+EM application showed the highest population of *Rhizobium* (35.1×10^6cfu g^{-1}), total bacteria (39.3×10^7 cfu g^{-1}), fungi (44.9×10^4 cfu g^{-1}) and Actinomycetes (41.7×10^3 cfu g^{-1}) surpassing the levels observed in the FUI control (Table 4). These findings are consistent with the research conducted by Kumar et al. (2021) and Sahu et al. (2015). The presence of yeast and yeast-like fungi in the soil is noteworthy due to their capability to produce various biologically active compounds, including phytohormones, vitamins, amino acids, and enzymes. These compounds play a pivotal role in stimulating plant growth and development, thereby contributing to increased productivity. Furthermore, the antimicrobial substances produced by yeast contribute to the mitigation of phytopathogenic infections, as emphasized by Yaduwanshi et al. (2021) and Sahu et al. (2023b).

4. Conclusion

Inoculation of microbial consortia of *Rhizobium, Pseudomonas* and EM culture improved the physico-chemical properties, availability of nutrients, microbial population and enzymatic activities (Phosphatase, Dehydrogenase) in soil that reflect better production of soybean. This treatment was followed by the Pseudo+EM. Overall, the inoculation of microbial consortia improved the soil fertility as well as productivity too instead of single inoculation.

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