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Effect of Microbial Inoculants on Plant Attributes and Nutrients Uptake by Soybean in Vertisols

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study was carried out during *kharif* season 2019-20 at the Research Farm, Department of Soil Science & Agricultural Chemistry, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh (INDIA), to assess the effect of microbial inoculants on plant attributes and nutrients uptake by soybean in Vertisols. The experiment was laid out under randomized block design (RBD) with three replications. The 15 treatments comprised of different beneficial microbial consortia in possible combinations applied as seed treatments. The crop was supplemented with recommended dose of fertilizers 20 N : 80 P_2O_5 : 20 K_2O kg ha^{-1}. Besides these, two control plots were maintained as fertilized un-inoculated control (FUI) and unfertilized un-inoculated control (UFUI). The findings revealed that the significant improvement were noticed by the application of consortia NPK+EM+PGPR in plant growth attributes of nodulation at 25, 45 & 65 DAS (71, 70 & 59% respectively), over control (9.5, 33.4 & 34.7 nodule $plant^{-1}$) and its biomass, (62, 69 & 74% respectively),over the control (0.58, 1.16 & 0.99 g $plant^{-1}$), plant height at 25, 45 & 65DAS were increased 61, 40, 41% respectively, over the control (16.20, 34.90 and 44.30 cm) and plant biomass, (48, 62 & 53%), over the control 1.67, 4.73 and 6.1 g $plant^{-1}$. Similarly, nutrient uptake (seed & stover)

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were also increased at 25, 45 and 65 stages of crop growth, with 36.6, 34.8 & 51.3% in seed and 66.7, 98.2 & 67.2% in straw respectively over the control (98.5, 63.8, 5.2, and 7.4, 24.9 and 44.4 kg ha^{-1} respectively). Thus, it may be concluded that the consortium of NPK + EM + PGPR was superior for sustainable crop production and soil health.

Keywords: Plant attributes; microbial inoculants; nutrients uptake; vertisols.

1. INTRODUCTION

Soybean [*Glycine max* (L.) Merri.] is an important leguminous oil seed crop and due to nutritional value, it has been considered as "Protein hope of future". Generally, soybean contains 40-45% protein and 18-20% oil [1]. The top two soybean growing states in India are Madhya Pradesh and Maharashtra with 45% and 40% shares of this crop, respectively. In Madhya Pradesh the soybean cultivation spreads over 5.2 M ha with total annual production of 6.7 M tones and productivity of 1285 kg ha^{-1} [2]. Soybean rhizosphere harbors vast proportions of soil microorganisms, whose activities largely determine the biological condition of the soil and influence the plant growth right from seed germination to maturity [3].

Different microbial consortia i.e. *Pseudomonas* as PGPR is the most efficient and effective strain with significant remarks on isolates of *P. fluorescens* and *P. putida* increasing growth and yield of different crops, especially legumes. *Rhizobium* (diazotroph) is a Gram- negative bacterium and symbiotic N_2-fixer with roots of legumes. It colonizes the roots of specific legumes to form tumor like growths called root nodules, which acts as the factories of ammonia production [4]. *Bacillus subtilis* a soil Gram-positive catalase-positive bacterium is known also as the hay bacillus or grass bacillus. The *Bacillus* sp. produces soluble exudates which is composed of five organic acids; gluconic acids, succinic acids, lactic acetic and propionic acids. The action of organic acids is recognized as a major mechanism responsible for the release of phosphates from the hydroxyl apatites [5]. *Rhodopseudomonas palustris* is a photrophic, rod-shaped, Gram-negative purple non-sulfur bacterium, having ability to switch between four different modes of metabolism [6]. Its role in degrading aromatic compounds has been in evidence extensively.

R. palustris utilizes a reductive coenzyme to convert via reduction and dehalogentation of 3-chlorobenzoate into acetyl-CoA and CO_2. The purple non-sulfuric bacteria can also perform N_2-fixation due to having unique genome encodes for three different nitrogen fixing enzymes. *Saccharomyces cerevisiae* is saprophytic, single celled egg-shaped yeast-fungi generally larger than most bacteria that grow on sugary solution, grapes etc. and known to contribute pleasant smell of bread. Soil yeast and yeast-like fungi produce a variety of biologically active compounds viz. phytohormones, vitamins, amino acids, enzymes etc. [7]. It has active stimulating effect on the plant growth, development and productivity. In addition, yeasts produce antimicrobial substances helping to reduce phytopathogenic infection.

Isolates of constitutional microorganisms of EM culture (Effective Microbial Culture) individually have already been evidenced beneficial but their consortium could be more valuable to augment the supply of nutrients through solubilization, anti-phytopathogenicity, induced phytoresistance and phytostimulator. In view of the above, the present investigation was carried out to find the effect of different microbial inoculants on plant attributes and nutrient uptake by soybean in a Vertisols.

2. MATERIALS AND METHODS

The present investigation was focused on effect of different microbial inoculants on nodule attributes, plant growth and nutrient uptake of soybean in Vertisols. The study was carried out during kharif season 2019-20 at the Research Farm, Department of Soil Science & Agricultural Chemistry, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur, Madhya Pradesh (INDIA). It is situated at 23°10'N latitude and 79°57" E longitude at 393 meters above the mean sea level.

The soil of the experimental site is Vertisol belonging to Kheri series of fine montmorillonitic hyperthermic family of T*ypic Haplusterts* with pH of 7.15, electrical conductivity 0.24 dS m^{-1} (1 : 2.5 soil : water ratio) and organic carbon 5.2 g

kg^{-1}. The soil available N, P and K were 226, 15.8 and 282 kg ha^{-1}, respectively. The soil available N was estimated by alkaline permanganate method [8], available P by Olsen's method [9] and available K by ammonium acetate extraction method [10]. At the start of the experiment, microbial population counts ware also analyzed by Serial Dilution Technique [11], which is presented in Table 1.

The experiment comprised 15 treatments with three replications having 45 plots (each plot 4 m × 2 m = 8 m^2) laid out under randomized block design (RBD). The treatments of different liquid biofertilizers either solo and/or consortia were applied on soybean (cv. JS-2069) as seed treatment / basal application as per the appropriate recommendations. The biofertilizers used were diazotroph (*Rhizobium*), PSB- Phosphate Solubilizing Bacteria (*Bacillus* sp.), KSB- Potash Solubilizing Bacteria (*Fraturia aurentia*), PGPR- Plant Growth Promoting Rhizobacteria (*Pseudomonas fluorescens*) and EM- Effective microbial culture/consortium (six bacteria, two fungus and one actinomycetes) culture. The recommended NPK dose for soybean, based on initial soil test, was 20:80:20 ($N : P_2O_5 : K_2O$ kg ha^{-1}). The sources of N, P and K used were urea, single super phosphate and muriate of potash. Besides these, two types of control plots were maintained as fertilized un-inoculated control (FUI) and unfertilized un-inoculated control (UFUI) to measure the comparative effects of different microbial inoculants. The treatment combinations are presented in Table 2.

2.1 Seed Treatment and Inoculation

Soybean seed was weighed 40 g separately for each plot in clean polythene bags. One ml of liquid formulation of each microorganism was transferred aseptically to previously sterilized appendage tubes (micro centrifuge tubes). Sterilized gum acacia (2%) was used as sticker solution and one ml of this solution was also transferred aseptically to micro centrifuge tubes separately. Seed treatment was done in shade and seeds were sown manually as early as possible. After germination, thinning was done to maintain the required plant population. Necessary plant protection measures were taken as per recommended package of practices [12].

Table 1. Initial microbial population counts of experimental soils

Particulars	Population Counts (cfu g^{-1})
Rhizobium	4.52×10^6
Phosphorous Solubilizing Bacteria - PSB	3.67×10^5
Potash Solubilizing Bacteria - KSB	3.91×10^7
Pseudomonasn sp. (PGPR)	5.43×10^7
Rhodopseudomonas sp.	1.27×10^3
Sachhromyces sp.	2.19×10^3
Streptomyces sp.	1.93×10^4
Aspergillus sp.	1.51×10^3
Lactobacillus sp.	2.14×10^3

Table 2. Details of different treatment combination

Treatment	Combination	Treatment	Combination
T1	*Rhizobium*	T9	NPK + PGPR
T2	NPK consortium	T10	PK + EM
T3	EM culture	T11	PK + PGPR
T4	PGPR	T12	NPK+EM+PGPR
T5	PK Consortium	T13	PK + EM + PGPR
T6	Rhizo +EM	T14	FUI
T7	Rhizo+ PGPR	T15	UFUI
T8	NPK + EM		

2.2 Growth Parameters

- **Nodulation attributes:** Nodulation studies were done at 25, 45 and 65 days of sowing by uprooting 3 plants plot^{-1} very carefully taking sufficient care to avoid any losses or damage of nodules. The rhizosphere soil was washed in the running water. After proper washing nodules of plants per plot were counted.
- **Fresh and oven dried weight of nodules (nodule biomass):** After counting, the nodules were detached from the roots and were kept in small paper bags. Then the nodule fresh weight was taken. Nodules were oven dried in hot air oven at 60^{0}C for (18-20 hrs) 3-4 days (till constant weight) to record their oven dried weight.
- **Plant height:** Plant height was measured at 25, 45, 65 DAS and at harvest. Three plants from each plot were taken and their heights were measured.
- **Plant dry biomass:** At 25, 45 and 65 DAS, the root portion of three plants were cut off and plants were dried in a hot air oven at 60^{0}C for 5-6 days (till constant weight) to record the dried shoot and root biomass. After recording the weight, the dried plant samples were ground in electric grinder for further analytical work.

2.3 Nutrients Uptake

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 [13], for phosphorus content of the plant extract was determined using Vanado Molybdo phosphoric yellow colour method [14] and potassium was determined with the help of flame photometer [14]. On the basis on nutrient content the nutrient uptake of soybean was calculated in kg ha^{-1} in relation to (dry matter production) yield ha^{-1}. All observations recorded on nodule attributes, crop growth and nutrients uptake were analyzed statistically [15].

3. RESULTS AND DISCUSSION

3.1 Plant Attributes

3.1.1 Nodulation of soybean at different growth stages

Studies on nodulation (nodule enumeration and its biomass) and the data on number of nodules and its oven dried weight per plant at 25, 45 and 65 DAS of crop growth stages have been presented in Table 3, which clearly indicated that the microbial consortium of NPK+EM+PGPR recorded significantly higher nodule number

Table 3. Effect of microbial inoculants on nodulation attributes of soybean at different growth stages

Treatment	Nodule enumeration (No. plant^{-1})			Nodule biomass (g plant^{-1})		
	25 DAS	45 DAS	65 DAS	25 DAS	45 DAS	65 DAS
Rhizobium	16.2	54.3	52.1	0.85	1.51	1.33
NPK consortium	13.8	47.2	46.1	0.73	1.45	1.43
EM culture	16.0	48.6	47.2	0.75	1.36	1.34
PGPR	12.2	38.4	37.1	0.57	1.52	1.22
PK consortium	12.6	38.2	37.1	0.59	1.36	1.06
Rhizo+EM	16.3	50.3	48.7	0.87	1.57	1.33
Rhizo+PGPR	15.6	50.7	49.1	0.80	1.55	1.34
NPK+EM	16.3	51.1	47.7	0.74	1.86	1.56
NPK+PGPR	16.0	50.6	48.7	0.65	1.61	1.31
PK+EM	15.2	47.2	45.6	0.78	1.71	1.41
PK+PGPR	16.7	49.8	49.4	0.73	1.90	1.57
NPK+EM+PGPR	17.1	56.4	55.3	0.94	1.96	1.72
PK+EM+PGPR	16.8	55.9	53.1	0.92	1.93	1.63
FUI	9.9	33.3	34.7	0.58	1.16	0.99
UFUI	8.2	30.7	28.2	0.49	1.04	0.74
Mean	15.1	46.8	45.4	0.73	1.56	1.33
CD5%	3.08	8.09	8.11	0.15	0.30	0.31

(17.1 nodules plant^{-1}) with an increment of 71% over FUI (9.9 nodules plant^{-1}) at 25 DAS. The nodule number at 45 DAS was increased from 30.7 to 56.4 nodules plant^{-1} with an average of 46.8 nodules plant^{-1}. The microbial consortium of NPK+EM+PGPR achieved significantly maximum nodules number (56.4 nodules plant^{-1}) with 69.6% increase over that of FUI (33.3 nodules plant^{-1}). Results further revealed that in 65 DAS was also counted maximum nodule number with the microbial consortium of NPK+EM+PGPR (55.3 nodule plant^{-1}) and 59% increase over FUI - Fertilized Un-Inoculated control (33.3 nodules plant^{-1}). The increase in nodulation might be due to synergistic effect of these microorganisms for biological nitrogen fixation as against their individuals [12]. Similar findings were also reported by [16] which clearly revealed that the number of nodules increased 30% by inoculation over un-inoculated.

The data of nodule biomass at 25 DAS revealed that the consortium of NPK+EM+PGPR registered significantly higher nodule biomass of 0.94 g plant^{-1} and 62% increment compared to that of FUI (0.58g plant^{-1}). The soybean crop at 45 DAS attained highest nodule biomass of 1.96 g plant^{-1} with an increment of 69% over that of FUI (1.16 g plant^{-1}) due to the treatment NPK+EM+PGPR. Further, the nodule biomass at 65 DAS, the consortium of NPK+EM+PGPR performed best for nodule biomass of 1.72 g plant^{-1} and 73.65% response relative to that of FUI (0.99 g plant^{-1}). Amule *et al.,* [17] also agreed with the findings that the consortia of bradyrhizobia and phosphate solubilizing bacteria (*Pseudomonas* sp.) as liquid inoculants on soybean enhanced the nodule number, fresh weight and dry weight of nodules. Similar findings have also been recorded by [12].

3.1.2 Plant height and its biomass at different Growth Stages of Soybean

Among all the treatments at 25 DAS, the consortium of NPK+EM+PGPR responded best for plant height of 26.1 cm and 61% increase over that of FUI (16.2 cm), followed by PK+EM+PGPR, PK+EM, NPK+EM and Rhizo+PGPR, with response of 50.1, 40.0, 33.9, and 33.1%, respectively (Table 4). Similar findings ware also reported at 45 and 65 DAS. It was reported that salicylic acid increases the cell division of apical meristem of seedlings and thereby improve plant growth. Effective microbial culture (Ems) enhance plant growth (plant height) and productivity by fixing atmospheric nitrogen and supplementing the plants with the fixed nitrogen as ammonia. Additionally, the release of trace elements, secreted antioxidant, exo-polysaccharides, bioactive compounds (vitamins, hormones and enzymes) by the EMs stimulate plant growth and productivity [18].

The plant biomass (Table 4) indicated that the consortium of NPK+EM+PGPR responded maximum for plant biomass of 2.47 g plant^{-1} and 47.9% increment over the FUI (1.67 g plant^{-1}). While the ensuing performing group was PK+EM+PGPR, NPK consortium, Rhizo+EM, EM culture and PK+PGPR with respective increment of 36.1, 34.1, 33.1, 32.7, and 30.9% at 45 DAS. Among all at 45 DAS, the consortium of NPK+EM+PGPR exhibited maximum plant biomass of 7.66 g plant^{-1} and response of 61.78% over that of FUI (4.73 g plant^{-1}). Further, at 65 DAS the treatment NPK+EM+PGPR performed best with maximum plant biomass of 9.35 g plant^{-1} and 53.30 % increase over that of FUI (6.10 g plant^{-1}). The higher plant biomass under this treatment combination was mainly due to more availability of N, P, K and S in soil for soybean plants. Moreover, growth promoting substances (phytohormones) are produced by these organisms which further promote plant biomass [19].

3.2 Nutrients Uptake by Soybean

The data pertaining to nutrient uptake by soybean was presented in Table 5 revealed that the nutrient uptake differs significantly with different treatment combinations. In seed, maximum uptake of N, P and K recorded under treatment receiving NPK+EM+PGPR was 138, 15.5 and 44.4 kg ha^{-1}, respectively. Applied NPK and EM with PGPR helped to increase the N uptake by soybean seed as compared to the application of PGPR alone (104, 12.3 and 33.8 NPK kg ha^{-1}, respectively). This might be attributed to potential of the endophytic actinomycetes that produces plant growth promoters used as co-inoculants with *Bradyrhizobium japonicum* to enhance the growth of soybean crop. These endophytes exhibited the potential to enhance plant growth, nitrogenase activity of root nodules and plant

nutrients uptake as well as the co-inoculation significantly increased the nutrient levels of nitrogen within a soybean plant [20]. Similar findings were also reported by [21& 22]. Further, NPK consortium gave the 127, 15.5 and 38.8 kg ha^{-1} NPK uptake, respectively. In stover the highest NPK uptake (100, 31.5 & 96.8 kg ha^{-1}, respectively) was recorded with NPK+EM+PGPR treatment combinations, followed by PK+EM+PGPR treatment combination gave 28.8 kg ha^{-1} P uptake and 91.6 kg ha^{-1} K uptake, while *Rhizobium* alone treatment gave 98 kg ha^{-1} N uptake.

Table 4. Effect of microbial inoculants on plant height and its Biomass at different growth stages of soybean

Treatment	Plant height (cm)			Plant Biomass (g $plant^{-1}$)		
	25 DAS	45 DAS	65 DAS	25 DAS	45 DAS	65 DAS
Rhizobium	16.7	36.3	42.4	2.17	5.19	8.07
NPK consortium	21.2	46.3	58.4	2.24	5.91	9.11
EM culture	22.1	40.4	52.4	2.22	6.68	9.13
PGPR	16.9	35.2	45.4	1.79	4.88	6.73
PK consortium	17.8	38.4	40.9	1.76	5.25	7.06
Rhizo+EM	21.1	46.1	57.3	2.23	6.58	9.22
Rhizo+PGPR	22.5	40.6	53.8	2.15	5.86	9.21
NPK+EM	23.1	43.3	58.7	2.16	6.98	9.14
NPK+PGPR	21.4	46.1	53.1	2.12	6.21	8.65
PK+EM	24.6	46.4	55.7	2.18	6.58	8.56
PK+PGPR	22.5	47.1	53.3	2.19	7.57	9.08
NPK+EM+PGPR	26.1	48.6	62.4	2.47	7.66	9.36
PK+EM+PGPR	24.8	48.0	60.0	2.28	7.23	9.26
FUI	16.2	34.8	44.3	1.67	4.73	6.10
UFUI	14.5	34.1	39.3	1.58	4.36	5.89
Mean	20.4	42.1	51.8	2.06	6.11	8.30
CD5%	4.2	7.4	9.9	0.43	1.29	1.57

Table 5. Effect of microbial inoculants on nutrients uptake (NPK) by soybean

Treatments	Nutrients Uptake (kg ha^{-1})								
	Seed			Stover			Total		
	N	P	K	N	P	K	N	P	K
Rhizobium	130	12.3	33.4	98	27.1	74.7	234	37.2	108.1
NPK consortium	127	15.4	38.8	88	25.6	83.6	216	41.3	122.5
EM culture	130	15.3	42.4	92	29.4	88.4	223	42.1	130.8
PGPR	104	12.3	33.8	68	21.3	60.7	173	30.5	94.6
PK consortium	103	12.1	32.3	61	20.3	89.2	175	37.3	121.5
Rhizo +EM	128	15.4	41.9	86	21.9	84.0	215	36.4	126.0
Rhizo +PGPR	124	14.4	39.1	85	23.2	75.2	210	33.9	114.4
NPK +EM	127	14.6	41.9	87	23.5	84.7	223	40.2	126.6
NPK+PGPR	133	15.2	43.6	91	24.7	86.6	225	39.3	130.2
PK+EM	127	14.8	41.6	85	22.7	84.5	212	37.1	126.2
PK+PGPR	126	14.8	40.8	84	22.4	82.8	211	38.3	123.6
NPK+EM+PGPR	138	15.5	46.0	100	31.5	96.8	240	44.1	142.9
PK+EM+PGPR	134	14.8	44.4	95	28.8	91.6	229	42.6	136.1
FUI	101	11.5	30.4	60	15.9	57.9	168	25.1	88.4
UFUI	83	9.7	24.8	55	17.1	42.7	139	19.3	67.5
Mean	121	13.9	38.3	83	23.7	78.8	207	36.3	117.3
CD5%	21.8	3.1	7.7	21.1	6.1	18.8	35	7.7	20.5

4. CONCLUSION

It was concluded that maximum number of nodules and its biomass was recorded with the consortium of NPK + EM + PGPR. This consortium also exhibited the best in term of plant height and its biomass and nutrients uptake (NPK) by seed and stover of soybean crop. The consortium of NPK + EM + PGPR was superior for N uptake by soybean crop and 36% response over FUI, while for P and K uptake by soybean crop, response of the consortium NPK + EM + PGPR over Fertilizer Un Inoculated (FUI) control were 44.6% and 51.3%, respectively.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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