Effect of Microbial Inoculants on Leghemoglobin Content in Nodules and Microbial Populations in Rhizosphere of Soybean in aVertisols

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 leghemoglobin content in nodules and microbial populations in rhizosphere of soybean in a Vertisols. The experiment was laid out under randomized block design with three replications. The fifteen treatments comprised of different beneficial microbial consortia in possible combinations applied as seed treatments. Besides these, two control plots were maintained as fertilized un-inoculated control (FUI) and unfertilized un-inoculated control (UFUI). The results revealed that the significant improvement were noticed by the application of consortia NPK+EM+PGPR in leghemoglobin content in nodules at 25, 45 & 65 DAS over control. Similarly, the microbial populations of the diazotroph, PSB, KSB, PGPR, phototroph, Lactic bacterium, actinomycetes, fungus and yeast were maximum in NPK+EM+PGPR treatment and followed by PK+EM+PGPR treatments. Thus, it may be concluded that the consortium of NPK + EM + PGPR was superior for batter nodulation and microbial population in rhizosphere of soybean in a Vertisol.

Keywords: Microbial inoculants; leghemoglobin; Microbial population; SoybeanVertisols.

INTRODUCTION

Soybean (*Glycine max* L.) is an important leguminous oil seed crop, which contains 40-45% protein and 18-20% oil. The top two soybean growing states in India are Madhya Pradesh and Maharashtra with 45% and 40% shares, 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} [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 [4]. It colonizes the roots of specific legumes to form tumor like growths called root nodules, which acts as the factories of ammonia production. *Bacillus subtilis* a soil Grampositive 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.*Frateuriaaurantia* is a potassium solubilizer which increases the potassium uptake by the plant.

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 study was conducted to find the effect of different microbial inoculants on leghemoglobin content and microbial populations in rhizosphere of soybean in a Vertisols.

Materials and Methods

The present investigation was conducted duringkharif 2019-20 under All India Network Project on Biofertilizers, to assess the effect of different microbial inoculants on leghemoglobin content in nodules and microbial populations in rhizosphere of soybean in aVertisolat the Research Farm, Department of Soil Science, 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 belonging to Kheri series of fine montmorillonitichyperthermic family of T*ypicHaplusterts*(Vertisol) 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 experiment comprised 15 treatments with three replications having 45 plots 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 (*Fraturiaaurentia*), PGPR- Plant Growth Promoting Rhizobacteria (*Pseudomonas fluorescens*) and EM- Effective microbial culture/consortium (six bacteria, two fungus and one actinomycetes) culture. The different treatment combinations are presented in Table 1.

Table 1: Details of different treatment combinations

TreatmentCombinations			
T_1	*Rhizobium*	**T_9**	NPK + PGPR
T_2	NPK consortium	**T_{10}**	PK + EM
T_3	EM culture	**T_{11}**	PK + PGPR
T_4	PGPR	**T_{12}**	NPK+EM+PGPR
T_5	PK Consortium	**T_{13}**	PK + EM + PGPR
T_6	*Rhizobium* +EM	**T_{14}**	FUI
T_7	*Rhizobium* + PGPR	**T_{15}**	UFUI
T_8	NPK + EM		

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 uninoculated control (FUI) and unfertilized uninoculated control (UFUI) to measure the comparative effects of different microbial inoculants.

Leghemoglobin content in nodules:-

In the present study, leghemoglobin content in nodules of soybean crop was estimated by the cyanmethemoglobin method. The principle of this method is the conversion of hemoglobin into cyanmethemoglobin by the addition of potassium cyanide and ferricyanide, whose absorbance is measured at 540 nm in a spectrophotometer against a standard solution. This method is considered as a reference method because it is accurate, cheap, sensitive to small variations [5].

Estimation of Microbial populations in rhizospheric soil:-

Samples of rhizospheric soil were used as fresh as possible without grinding, sieving or any modifications. The collected samples were kept in low density polyethylene bags and could be stored in refrigerator at 4^{o}C.At the start of the experiment, microbial population counts ware analyzed by Serial Dilution Technique [6].The initial microbial population counts of experimental soils presented in Table 2.

Table 2: Initial microbial population counts of experimental soils

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

Statistical analysis:-

The data generated on leghemoglobin content in soybean nodulesand microbial populations in rhizospheric soil were statistically analyzed to draw suitable inference as per standard method [7].

RESULT AND DISCUSSION

Leghemoglobin content in soybean nodules:-

The leghemoglobin content was determined in the soybean root nodules at 25, 45 and 65 DAS and presented in Table 3.At 25 DAS of soybean, the leghemoglobin content in nodules varied from 2.10 to 3.31 mg g^{-1} nodule with a mean value of 2.74 mg g^{-1} nodule. The maximum leghemoglobin content was estimated with the treatment NPK+EM+PGPR of soybean nodules (3.31 mg g^{-1} fresh nodule weight) which was 45.7% more as compared to that of FUI (2.27 mg g^{-1} fresh nodule weight), followed by PK+EM+PGPR, Rhizo+PGPR, Rhizo+EM, NPK+EM, EM culture, NPK+PGPR, NPK consortium and *Rhizobium* for leghemoglobin content of 3.29, 3.26, 3.18, 3.07, 2.89, 2.89, 2.74 and 2.71 mg g^{-1} nodule, respectively corresponding to increment of 44.9, 43.6, 40.2, 35.2, 27.2, 27.2, 20.7 and 19.2%, respectively over that of FUI.

Similarly, the results at 45 DAS and 65 DAS of soybean showed that the application of NPK+EM+PGPR contributed to the maximum leghemoglobin content of 3.76 and 3.11 mg g^{-1} nodule with response of 38 and 50%, respectively over that of FUI, it could bedue to the better root and nodules development [8, 9].

Microbial populations (*Rhizobium*, PSB, KSB and PGPR) in rhizosphere of soybean at harvest

The population counts of *Rhizobium* in rhizosoheric soil of soybean at harvest are presented in Table 4.It is apparent from results that the *Rhizobium* population were maximum of 6.48 log cfu (30.19 $x10^5$ cfu g^{-1} soil) with the inoculation of consortium NPK+EM+PGPR along with the relative response of 1.85 log fold increase over that of control FUI (3.51 log cfu = $32.35x10^2$cfu g^{-1} soil). This was followed by the response of PK+EM+PGPR, *Rhizobium*, Rhizo+EM, Rhizo+PGPR, NPK consortium, NPK+EM, PK+PGPR,PK consortium and PK+EM for the diazotrophic population of 6.44 log cfu ($27.54x10^5$ cfu g^{-1} soil), 5.93 log cfu ($85.11x10^4$ cfu g^{-1} soil), 5.86 log cfu ($72.44x10^4$ cfu g^{-1} soil), 5.71 log cfu ($51.28x10^4$ cfug^{-1} soil), 5.69 log cfu ($48.97x10^4$ cfu g^{-1} soil), 5.69 log cfu ($48.97x10^4$ cfu g^{-1} soil), 5.61 log cfu ($40.73x10^4$ cfug^{-1} soil), 5.42 log cfu ($26.30x10^4$ cfu g^{-1} soil), 5.14 log cfu ($13.80x10^4$ cfu g^1 soil) and 5.13 log cfu ($13.37x10^4$ cfug^{-1} soil), respectively with the respective response of 1.84, 1.69, 1.67, 1.63, 1.62, 1.60, 1.54 and 1.46 log fold increase over that of FUI.The population of soybean rhizobia in rhizospheric soil was also noted more with 80 kg P_2O_5+LRh by 1.69, 1.55 and 1.57 log folds, respectively over to that of control (3.297, 5.499 and 4.619 log cfu g^{-1} soil at 21 DAS, 45 DAS and at harvest, respectively [10, 11].

The data on populations of PSB (*Bacillus* sp.) in rhizospheric soil of soybean at harvest are presented in Table 4.The consortium of NPK+EM+PGPR increased the maximum microbial population maximum for the PSB counts of 9.81 log cfu ($64.56x10^8$ cfu g^{-1} soil) and increment of 1.38 log fold over that of FUI (log cfu7.11 = $12.88x10^6$ cfu g^{-1} soil).The findings showed that the seed treatment with *A. awamori* increased fungal (25.25 and 29.06 cfu x 10^3 /g) and actinomycetes population (23.44 and 26.19 cfu x 10^8/g) while *B. polymixa* increased phosphate solubilizing bacterial counts (21.37 and 23.31 cfu x 10^6/g) significantly at 30 and 60 DAS, respectively. The application of FYM (5 t/ha) had significantly increased the fungi (22.21 and 27.25 cfu x 10^3/g), actinomycetes (20.37 and 23.77 cfu x 10^8 /g), bacterial (30.55 and 36.02 cfu x 10^6/g) and PSB population (18.42 and 21.30 cfu x 10^6 /g) in the soybean field at 30 and

60 DAS, respectively[12, 13]. Inoculation of NPK+EM+PGPR might have given added advantages over native microbial population [14, 15].

The data pertaining to the population of KSB (*F. aurantia*) in soil of soybean at harvest were counted and listed in Table 4. The consortium of NPK+EM +PGPR was found statistically superior to increase KSB population of 7.42 log cfu ($26.30x10^6$ cfu g^{-1} soil) along with the relative response of 1.93 log fold increase over that of FUI (3.85 log cfu = $70.79x10^2$cfu g^{-1} soil). This was followed by the response of PK+EM+PGPR, PK+PGPR, PK+EM, PK consortium, NPK+PGPR, Rhizo+PGPR, NPK consortium, NPK+EM and EM culture for the microbial population of 7.24 log cfu ($17.37x10^6$ cfu g^{-1} soil), 6.80 log cfu ($63.09x10^5$ cfu g^{-1} soil), 6.74 log cfu ($54.95x10^5$ cfu g^{-1} soil), 6.73 log cfu ($53.70 x10^5$ cfug^{-1} soil), 6.53 log cfu ($33.88x10^5$ cfu g^{-1} soil),), 6.44 log cfu ($27.54 x10^5$ cfu g^{-1} soil), 6.31 log cfu ($20.41x10^5$ cfu g^{-1} soil), 5.93 log cfu ($85.11x10^4$ cfu g^{-1} soil) and 5.25 log cfu ($17.78x10^4$ cfu g^{-1} soil), respectively and the respective responses of 1.88, 1.77, 1.75, 1.70, 1.67, 1.64, 1.54, 1.36 and 1.28 log fold increase over that of FUI [16, 17].

The data on population of PGPR(*P. fluorescens*) in soil (0-15cm) of soybean revealed that the consortium of NPK+EM+PGPR were recorded (Table 4) maximum population of PGPR for 9.66 log cfu ($45.70x10^8$ cfu g^{-1} soil) with the relative response of 1.57 log fold increase than that of control FUI (6.16 log cfu = $14.45x10^5$ cfu g^{-1} soil), followed by the PK+EM+PGPR1.56log fold increase over that of FUI [18].*Pseudomonas* population in soil ranged from 6.5 to $8.02x10^4$cfu g^{-1} of soil, maximum was influenced by seed inoculation with *Pseudomonas* at 3 g kg^{-1}[19]. It might be attributed to the synergistic effect of the microorganism's present consortium NPK+EM+PGPR contributing to higher PGPR population at harvest. *Pseudomonas* are the most efficient and effective strain that can act as PGPR. *P. fluorescens* has simple nutritional requirements and grows well in mineral salts media supplemented with any of a large number of carbon sources [20].

Microbial populations of EM culture (*Rhodopseudomonas* sp., *Lactobacillus* sp., *Streptomyces* sp., *Aspergillus* sp.and *Saccharomyces*) in soil at harvestof soybean

The data on population of phototroph*Rhodopseudomonas*sp. (one of EM organism) in soil at harvest of soybean cropare listed in Table 5.It was noticed that the population of the phototrophic bacteria was enumerated maximum of 5.79 log cfu ($61.65x10^4$ cfu g^{-1} soil) with the relative response of 1.85 log fold increase with inoculation of consortium of PK+EM+PGPR over that of FUI (log cfu 3.13 = $1.34x10^3$

cfu g^{-1}soil).The inoculation of PPB (Phototrophic Purple Bacteria:*Rhodopseudomonaspalustris*) increased populations of PPB in the plots, significantly increased by the inoculation. This might be attributed due to the ability of *R. palustris* to degrade aromatic compounds has been researched extensively. *R. palustris* utilizes a reductive coenzyme to convert, *via* reduction and dehalogentation, 3-chlorobenzoate into acetyl CoA and carbon dioxide [21, 22].

Data pertaining to the population of lactic bacteria *Lactobacillus* sp. (one of EM culture) in rhizosphere of soybean revealed that the consortium of NPK+EM+PGPR recorded (Table5) maximum microbial population of 5.53 log cfu ($33.88x10^4$ cfu g^{-1} soil) with the relative response of 2.07 log fold increase as compare to that of FUI (2.67 log cfu = $4.67x10^2$cfu g^{-1} soil). This was followed by the response from PK+EMand PK+EM+PGPR for 5.08 log cfu ($12.02x10^4$ cfu g^{-1} soil) and 4.94 log cfu ($87.06x10^3$ cfu g^{-1} soil), respectively along with the response of 1.90 and 1.85log fold increase over that of FUI. The numbers of bacteria, fungi and actinomycetes has increased after the soil was treated with EM cultures. EM cultures markedly increased the numbers of *Enterobacter* sp. and starch digesting bacteria over that of unfertilized [23].

The populations of actinomycetes *S. badius* (one of EM culture) in soil of soybean at harvest are exhibited in Table 5. The consortium of NPK+EM+PGPR achieved maximum population ofthe actinomycetesfor 4.64 log cfu ($43.65x10^3$ cfu g^{-1} soil) with the relative response of 1.66 log fold increase over that of control FUI (2.76 log cfu = $6.16x10^2$cfu g^{-1} soil). Similarly, the actinomycetes population in soil from most of the plots sampled was about $5x10^5$cfu g^{-1} of dry [24, 25]. The seed inoculation with *Pseudomonas* at 3 g kg^{-1} showed the maximum number of actinomycetes counts $11.4x10^5$ cfu g^{-1} soil that was 13.9% more actinomycetes population over that of control. All the treatments showed numerical increase in actinomycetes population in comparison to that of control.

The population of fungus *Aspergillus* sp.(one of EM culture) in rhizosphere of soybean at harvest are shown in Table 5. It is apparent from the results that the consortium of NPK+EM+PGPR recorded the maximum population of *A. niger*by 4.28 log cfu ($19.05x10^3$ cfu g^{-1} soil) with the relative response of 1.66 log fold increase over the control FUI 2.58 log cfu ($3.80x10^2$cfu g^{-1} soil).The fungus *A. niger* grows aerobically on organic matter as a common contaminant. It is known to remove the heavy metals like lead, cadmium and copper from the water [26. 27].

The results clearly showed that the population of yeast *S. cerevisiae* (one of EM culture) in soil of soybean at harvest was increased from 3.12 log cfu (1.31 $x10^3$cfu g^{-1}soil) to 6.16 log cfu (14.45$x10^5$cfu g^{-1}soil) with mean value of 4.82 log cfu (66.06$x10^3g^{-1}$soil). The consortium of NPK+EM+PGPR boosted the population of yeast to maximum by 6.16 log cfu (14.45$x10^5$ cfu g^{-1} soil) with the relative response of 1.60 log fold increase over that of control FUI (3.85 log cfu = 7.07$x10^3$cfu g^{-1} soil), followed by the performance of PK+EM+PGPR and EM culture for 6.03 log cfu (10.71$x10^5$ cfu g^{-1} soil) and 5.96 log cfu (91.20$x10^4$ cfu g^{-1} soil), respectively corresponding to the increment of 1.27 and 1.55log fold over that of FUI.

Soil yeast and yeast-like fungi produce a variety of biologically active compounds (phytohormones, vitamins, amino acids, enzymes etc.) that have active stimulating effect on the plant growth and development and help to increase their productivity. In addition, yeasts produce antimicrobial substances helping to reduce phytopathogenic infection. *Rhizobium*, *Pseudomonas* spp., *Bacillus subtilis*, *Frateuriaaurantia* and effective microorganisms (EM culture) isolates individually are found beneficial but their consortium could be more valuable resource to augment the supply of nutrients through solubilization or mobilization of nutrients [28].

CONCLUSION

It was concluded that the microbial populations of the diazotroph, PSB, KSB, PGPR, phototroph, Lactic bacterium, actinomycetes, fungus and yeastwere maximum in NPK+EM+PGPRtreatment and followed by PK+EM+PGPR treatments.Microbes of EM culture enhance plant growth and productivity by fixing atmospheric nitrogen and supplementing the plants with the fixed nitrogen as ammonia. Additionally, the release of trace elements, secreted antioxidants, exo-polysaccharides, bioactive compounds (vitamins, hormones and enzymes) by the EMs stimulated plant growth and productivity.

REFERENCES

1. SOPA. The soybean Processors Association of India. Email: sopa@sopa.org;2018.
2. Khandagle, A.; Dwivedi, B.S.; Dwivedi, A.K.; Panwar, S.; Thakur, R.K. (2020). Nitrogen fractions under long-term fertilizer and manure applications in soybean – wheat rotation in a Vertisol. *Journal of the Indian Society of Soil Science*, 68, 186-193.

3. Awasthi R, Tewari R, Nayyar H. Synergy between plants and P solubilizing microbes in soils: effects on growth and physiology of crops. International Research Journal of Microbiology. 2011;2(12):484-503.
4. Sawarkar S.D,Thakur Risikesh and Khamparia R.S. (2010). Impact of Long Term Continuous Use of Inorganic and Organic Nutrients on Micronutrients Uptake by Soybean in Vertisol. *Journal of Soils and Crops*, **20** (2): 207 – 210.
5. Wilson DO and Reisenauer HM. 1963. Determination of leghaemoglobin in legume nodules. Analytical Biochemistry, 6(1), pp.27-30.
6. David A and Davidson CE. 2014. Estimation method for serial dilution experiments. Journal of Microbiological Methods, 107, pp 214-221.
7. Panse, V.G. and S.V. Sukhatme (1970). Statistical methods for Agril. Workers. ICAR Publication.
8. Thakur R.K., N.K. Bisen, A.K. Shrivastava, S.K. Rai and S. Sarvade (2023). Impact of Integrated Nutrient Management on Crop Productivity and Soil Fertility under Rice (*Oryza Sativa*) – Chickpea (*Cicer Arietinum*) Cropping System in Chhattisgarh Plain Agro-Climatic Zone. *Indian Journal of Agronomy*, 68(1): 9-13.
9. Eylenbosch D, Dumont B, Baeten V, Bodson B, Delaplace P and Pierna JAF. 2018. Quantification of leghaemoglobin content in pea nodules based on near infrared hyperspectral imaging spectroscopy and chemometrics. Journal of Spectral Imaging, 7.
10. Tiwari, R.; Dwivedi, B.S.; Sharma, Y.M.; Thakur, R.; Sharma, A.; Nagwanshi, A. (2023). Soil Properties and Soybean Yield as Influenced by Long Term Fertilizer and Organic Manure Application in a Vertisol under Soybean-Wheat Cropping Sequence. *Legume Research*. DOI: 10.18805/LR-5111.
11. Dwivedi, B.S., Rawat, A.K., Dixit, B.K. and Thakur, R.K. (2016) Effect of inputs integration on yield, uptake and economics of kodo millet (*Paspalumscrobiculatum* L). *Economic Affairs*, **61**(3): 519-526.
12. Meena, Suresh and Ghasolia P. 2013. Effect of phosphate solubilizers and FYM on microbial population of soybean field [*Glycine Max* (L.) Merrill]. The Bioscan, 8(3), pp.965-968.
13. Thakur Risikesh and Sawarkar S.D. (2009). Influence of long term continuous application of nutrients and spatial distribution of sulphur on soybean-wheat cropping sequence. *Journal of Soils and Crops***19**, 225 – 228.
14. Yaduwanshi B, Sahu RK, Mitra NG, Amule FC and Jakhar S. 2019. Effect of Microbial Consortia on Growth, Nodulation, Yield and Nutrient Uptake of Soybean in Vertisol of Central India. Int. J. Curr. Microbiol. App. Sci, 8(9), pp.2649-2659.
15. Sharma Y.M., Jatav R.C., Sharma, G.D. and Thakur Risikesh (2013). Status of Micronutrients in Mixed Red and Black Soils of Rewa District of Madhya Pradesh, India. *Asian Journal of Chemistry*, 25 (6) : 3109-3112.
16. Bairwa Jalendra, B.S. Dwivedi, Anay Rawat, R.K. Thakur and Neeta Mahawar (2021). Long-term effect of nutrient management on soil microbial properties and

nitrogen fixation in a Vertisol under soybean–wheat cropping sequence. *Journal of the Indian Society of Soil Science*, 69(2): 171-178.

17. Thakur Risikesh,Sawarkar S.D., Kauraw D.L. and Singh Muneshwar (2010). Effect of Inorganic and Organic Sources on Nutrients Availability in a Verisol. *Agropedology*, **20** (1): 53-59.
18. Kushwaha, S.; Sawarkar, S.D.; Thakur, R.; Khamparia, N.K.; Singh, M. (2017). Impact of Long-Term Nutrient Management on Soil N dynamics under Soybean – Wheat Cropping Sequence on a Vertisol. *Journal of the Indian Society of Soil Science*, 65, 274-282.
19. Pathariya, Priyanka, Dwivedi, B.S, Dwivedi, A.K., Thakur, R.K., Singh Muneshwar and Sarvade, S. (2022) Potassium Balance under Soybean–wheat Cropping System in a 44 Year Old Long Term Fertilizer Experiment on a Vertisol, *Communications in Soil Science and Plant Analysis*, **53**(2): 214-226.
20. Patel Gajendra, Dwivedi BS, Dwivedi AK, Thakur Risikesh and Singh Muneshwar (2018).Long-term Effect of Nutrient Management on Soil Biochemical Properties in a Vertisol under Soybean–Wheat Cropping Sequence. *Journal of the Indian Society of Soil Science*, 66 (2): 215-221.
21. Harada N, Nishiyama M, Otsuka S and Matsumoto S. 2005.Effects of inoculation of phototrophic purple bacteria on grain yield of rice and nitrogenase activity of paddy soil in a pot experiment. Soil Science & Plant Nutrition, 51(3):361-7.
22. Dubey Lokesh, Dwivedi BS, Dwivedi AK and Thakur RK (2016) Effect of long term application of fertilizers and manure on profile distribution of various phosphorus fractions in Vertisol. *Green Farming*, **7**(2): 365-370.
23. Meshram MK, Dwivedi BS, Naik KR, Thakur RK and Keram KS (2018) Impact of organic and inorganic sources of nutrients on yield, nutrient uptake, soil fertility and economic performance of rice in a TypicHaplustert. *Journal of Soils and Crops*, **28**(1) : 31-36.
24. Thakur Risikesh, S. Sarvade and B.S. Dwivedi (2022). Heavy Metals: Soil Contamination and Its Remediation. *AATCC Review*, 10(02): 59-76.
25. Sahur A, Ala A, Patandjengi B and Syamun E. 2018. Effect of Seed Inoculation with Actinomycetes and *Rhizobium* Isolated from Indigenous Soybean and Rhizosphere on Nitrogen Fixation, Growth, and Yield of Soybean. International Journal of Agronomy, 2018.
26. Khatik S.K., Thakur Risikesh and Sharma G.D. (2006). Lead: The Heavy Metal in Soil, Water and Plant Environment. *Journal of Industrial Pollution Control*, 22(2): 233–244.
27. Thakur Risikesh, Sharma G.D., Dwivedi B.S. and Khatik S.K. (2007). Chromium: As a Pollutant. *J. of Industrial Pollution Control*, 23(**2**): 197-203.
28. Keram K.S., Sharma B.L., Sharma G.D. and Thakur R.K. (2014). Impact of zinc application on its translocation into various plant parts of wheat in a Vertisol. *The Bioscan* 9(2): 491-495.

Table 3: Effect of microbial inoculants on leghemoglobin content in nodules of soybean at different growth stages

Treatment	Leghemoglobin content in nodules (mg g^{-1} nodule)		
	25 DAS	**45 DAS**	**65 DAS**
Rhizobium	2.75	3.19	2.54
NPK consortium	2.74	3.19	2.54
EM culture	2.89	3.34	2.69
PGPR	2.21	2.66	2.01
PK consortium	2.30	2.75	2.10
Rhizobium +EM	3.18	3.63	2.98
Rhizobium +PGPR	3.26	3.71	3.06
NPK +EM	3.07	3.52	2.87
NPK+PGPR	2.89	3.34	2.69
PK+EM	2.63	3.08	2.43
PK+PGPR	2.65	3.10	2.45
NPK+EM+PGPR	3.31	3.76	3.11
PK+EM+PGPR	3.29	3.74	3.09
FUI	2.27	2.72	2.07
UFUI	2.10	2.55	1.90
SE_m ±	**0.16**	**0.16**	**0.16**
CD (*p=0.05*)	**0.46**	**0.46**	**0.46**

Table 4 :Effect of microbial inoculants on microbial populations of *Rhizobium*, PSB, KSB and PGPR in rhizosphere of soybean at harvest

Treatment	Population of microorganism [log cfu and in parenthesis cfu g^{-1} soil]			
	Diazotroph (*Rhizobium* sp.)	**PSB (*Bacillus* sp.)**	**KSB (*Fraturia* sp.)**	**PGPR (*Pseudomonas* sp.)**
Rhizobium	5.71 ($51.28x10^4$)	7.17 ($14.79x10^6$)	4.92 ($83.17x10^3$)	7.04 ($10.9x10^6$)
NPK consortium	5.86 ($72.44x10^4$)	7.75 ($56.23x10^6$)	6.31 ($20.41x10^5$)	6.96 ($91.20x10^6$)
EM culture	4.62 ($41.68x10^3$)	7.51 ($32.35x10^6$)	5.25 ($17.78x10^4$)	7.93 ($85.11x10^6$)
PGPR	4.61 ($40.73x10^2$)	7.37 ($23.44x10^6$)	4.80 ($63.09x10^3$)	9.57 ($37.15x10^8$)
PK consortium	5.61 ($40.73x10^4$)	8.79 ($61.65x10^7$)	6.73 ($53.70x10^5$)	8.73 ($53.70x10^7$)
Rhizobium+EM	5.14 ($13.80x10^4$)	8.20 ($15.84x10^7$)	4.66 ($45.70x10^3$)	8.20 ($15.84x10^7$)
Rhizobium+PGPR	5.42 ($26.30x10^4$)	8.31 ($20.41x10^7$)	6.44 ($27.54x10^5$)	8.87 ($74.13x10^7$)
NPK +EM	5.69 ($48.97x10^4$)	8.95 ($89.12x10^7$)	5.93 ($85.11x10^4$)	8.11 ($12.88x10^7$)
NPK+PGPR	5.13 ($13.37x10^4$)	8.86 ($72.44x10^7$)	6.53 ($33.88x10^5$)	8.96 ($91.20x10^7$)
PK+EM	5.93 ($85.11x10^4$)	9.04 ($10.96x10^8$)	6.74 ($54.95x10^5$)	8.99 ($97.72x10^7$)
PK+PGPR	5.69 ($48.97x10^4$)	9.51 ($35.48x10^8$)	6.80 ($63.09x10^5$)	9.27 ($18.62x10^8$)
NPK+EM+PGPR	6.48 ($30.19x10^5$)	9.81 ($64.56x10^8$)	7.42 ($26.30x10^6$)	9.66 ($45.70x10^8$)
PK+EM+PGPR	6.44 ($27.54x10^5$)	9.32 ($20.89x10^8$)	7.24 ($17.37x10^6$)	9.61 ($40.73x10^8$)
FUI	3.51 ($32.35x10^2$)	7.11 ($12.88x10^6$)	3.85 ($70.79x10^2$)	6.16 ($14.45x10^5$)
UFUI	3.08 ($12.02x10^2$)	6.51 ($32.35x10^5$)	2.62 ($41.60x10^1$)	5.79 ($61.65x10^4$)
SE_m ±	**0.41**	**0.39**	**0.60**	**0.38**
CD (*p=0.05*)	**1.14**	**1.13**	**1.20**	**1.10**

Table 5: Effect of microbial inoculants on population of microorganisms of EM culture in soil of soybean at harvest

Treatment	Population of microorganism [log cfu and in parenthesis cfu g^{-1} soil]				
	Phtotroph ***R. palustris***	**Lactic bacterium** ***L. lactis***	**Actinomycetes** ***S. badius***	**Fungus** ***A. niger***	**Yeast** ***S. cerevicae***
Rhizobium	3.26 ($1.81x10^3$)	2.62 ($4.16x10^2$)	2.83 ($6.76x10^2$)	2.66 ($4.57x10^2$)	3.88 ($7.58x10^3$)
NPK consortium	3.14 ($1.38x10^3$)	2.91 ($8.12x10^2$)	3.01 ($1.04x10^3$)	2.77 ($5.88x10^2$)	4.07 ($11.74x10^3$)
EM culture	3.58 ($3.80x10^3$)	4.34 ($21.87x10^3$)	4.18 ($15.13x10^3$)	3.83 ($6.76x10^3$)	5.96 ($91.20x10^4$)
PGPR	3.05 ($1.12x10^3$)	2.88 ($7.58x10^2$)	3.29 ($1.94x10^3$)	2.97 ($9.33x10^2$)	3.78 ($6.02x10^3$)
PK consortium	3.24 ($1.73x10^3$)	2.96 ($9.12x10^2$)	3.35 ($2.23x10^3$)	3.46 ($2.88x10^3$)	4.17($14.79x10^3$)
Rhizobium +EM	4.43 ($26.91x10^3$)	4.12 ($13.18x10^3$)	4.19 ($15.48x10^3$)	3.65 ($4.46x10^3$)	5.80 ($63.09x10^4$)
Rhizobium +PGPR	4.08 ($12.02x10^3$)	4.03 ($10.71x10^3$)	3.70 ($5.01x10^3$)	3.18 ($1.51x10^3$)	4.60 ($39.81x10^3$)
NPK +EM	3.99 ($9.77x10^3$)	4.04 ($10.96x10^3$)	4.31 ($20.41x10^3$)	3.93 ($8.51x10^3$)	5.47 ($29.51x10^4$)
NPK+PGPR	4.08 ($12.02x10^3$)	4.23 ($16.98x10^3$)	3.23 ($1.69x10^3$)	3.59 ($3.89x10^3$)	4.67 ($46.77x10^3$)
PK+EM	5.44 ($27.54x10^4$)	5.08 ($12.02x10^4$)	4.25 ($17.78x10^3$)	4.10 ($12.58x10^3$)	5.78 ($60.25x10^4$)
PK+PGPR	5.14 ($13.80x10^4$)	4.59 ($38.90x10^3$)	4.16 ($14.45x10^3$)	3.99 ($9.77x10^3$)	4.94 ($87.09x10^3$)
NPK+EM+PGPR	5.58 ($38.01x10^4$)	5.53 ($33.88x10^4$)	4.64 ($43.65x10^3$)	4.28 ($19.05x10^3$)	6.16 ($14.45x10^5$)
PK+EM+PGPR	5.79 ($61.65x10^4$)	4.94 ($87.06x10^3$)	4.63 ($42.65x10^3$	4.15 ($14.12x10^3$)	6.03 ($10.71x10^5$)
FUI	3.13 ($1.34x10^3$)	2.67 ($4.67x10^2$)	2.76 ($6.16x10^2$)	2.58 ($3.80x10^2$)	3.85 ($7.07x10^3$)
UFUI	2.61 ($4.07x10^2$)	2.29 ($1.94x10^2$)	2.55 ($3.54x10^2$)	2.45 ($2.81x10^2$)	3.12 ($1.31x10^3$)
SE_m ±	**0.23**	**0.25**	**0.23**	**0.23**	**0.29**
CD (*p=0.05*)	**0.67**	**0.72**	**0.66**	**0.66**	**0.84**