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Impact of Plant Growth Promoting Rhizobacteria in Restoring Soil and Crop Attributes

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ABSTRACT

Background: To evaluate the effect of selected plant growth promoting rhizobacteria (PGPR) (*Paenibacillus polymyxa, Pseudomonas putida* and *Azospirillum brasilense*) and *Trichoderma* sp. in the restoration of selected attributes of soil fertility and the crop productivity in Indian Vindhyan semi-arid region.

Methods: An experiment was conducted at the Rajiv Gandhi South Campus, Banaras Hindu University, with test crop brinjal (*Solanum melongena* L.). Fourteen treatments: four mono-inoculants (T1 to T4), six bi-inoculants (T5 to T10), two tri-inoculants (T11 and T12), one tetra-inoculant (T13) and control (T14) were included in the present study. *Paenibacillus polymyxa*, *Pseudomonas putida*, *Trichoderma* sp. and *Azospirillum brasilense* were used in single or in combination in all treatments.

Result: The results showed a positive increment in organic C and total N in all treatments. This increase was maximum in T13 (*Paenibacillus polymyxa + Pseudomonas putida + Trichoderma* sp. + *Azospirillum brasilense)* treatment followed by T11> T12> T8> T9> T5> T6> T10> T2> T3> T4> T1 compared to control. Positive increment in plant height, number of leaves and flowers were also noted in T13, T11 and T12 treatments. Maximum above-ground biomass and below-ground biomass were recorded in tetra inoculant treatment. Improved nutrient acquisition in T13 (tetra-inoculant) and T11 and T12 (tri-inoculant) treatments is due to increasing nutrient uptake of N from nitrogen-fixing bacteria and uptake of P from phosphate mineralizing. Thus, for restoration of selected soil and crop attributes through PGPR and *Trichoderma* sp. especially through tetra-inoculants or tri-inoculantsis considered to be a good technique.

Key words: Crop growth, Degradation, PGPR, Restoration, Soil fertility, Sustainability.

INTRODUCTION

The semi-arid soil of the Indian Vindhyan region is degrading because of various reasons like drought, soil erosion, deforestation, desertification *etc.* Globally, land degradation negatively impacts about 3.2 billion people depending directly or indirectly on this resource. For fulfilling the requirement of several sustainable development goals (SDGs), like SDG 2 (Zero hunger), SDG 13 (Climate Action), SDG 15 (Life on land) and SDG 17 (Partnerships for the Goals), it is important to reverse the effect of degradation. So, the restoration of ecosystem attributes is important. It was reported that nutrient use efficiency, N uptake and productivity in dryland agroecosystem can be enhanced by the application of multipurpose tree leaves (Srivastava and Singh, 2013; 2019; Srivastava, 2019). They are known to improve plant growth in many ways when compared to synthetic fertilizers, insecticides and pesticides (Saha *et al.,* 2020; Sani *et al.,* 2020). Many Rhizobacteria and fungal species, archaea, fungi, algae, insects, annelids and other invertebrates can be utilized as Plant Growth Promoting Rhizobacteria (PGPR) or biofertilizers (Zhou *et al.,* 2016; Singh *et al.,* 2020). The implication of PGPR for restoring agricultural land sustainability have gained worldwide importance, as now the emphasis has shifted from chemical to biological approach in the agricultural system (Srivastava and Singh, 2017; Oleńska *et al.,* 2020).

The direct mechanisms of PGPR include biological nutrient uptake and solubilization (Oleńska *et al.,* 2020), nitrogen fixation, phosphate solubilization (Yadav *et al.,* 2014), siderophore production and iron uptake (Zhou *et al.,* 2016) and phytohormone synthesis. In past, various researches have been carried to study the effect of rhizospheric bacteria on the growth of different crops including legumes, vegetables and spices (Verma *et al.,* 2014; Sellitto *et al,* 2019). There is a scarcity of information about the application of PGPR for the restoration of soil fertility and crop productivity in the semi-arid Vindhyan region. In this view, the current study was carried out to evaluate the effect of PGPR and *Trichoderma* sp. alone and in combinations with each other's on the restoration of selected attributes of soil fertility (like soil organic carbon, nitrogen) and the crop productivity (test crop-brinjal) in Indian Vindhyan semi-arid region.

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MATERIALS AND METHODS

The experiment was carried out at the Rajiv Gandhi South Campus, Banaras Hindu University, Barkachha, Mirzapur which is situated in the Vindhyan region of district Mirzapur (25°10′ latitude, 82°37′ longitude and altitude of 427 meters above mean sea level). The soil of this region is sandy loam red laterite in texture with low drainage made from Vindhyan rocks, invariably poor fertility status. The soil is slightly acidic in reaction (5-6 pH), poor in nitrogen as well as phosphorus and moderate in potash. The soil had a poor amount of organic carbon below 0.5% (Srivastava and Singh, 2002; Srivastava *et al.,* 2020).

The pot experiments were conducted over one crop period (from December 2018-April 2019) with the incorporation of PGPR of Siliguri variety. The test crop selected for the experiment was brinjal (order Sonales and family Solanaceae) because of its versatile nature and adaptation to different agro-climatic regions. Four sterilized seeds of brinjal were taken separately in the test tube and added total volume of 5ml broth cultures of microbial consortia. After this, same four seeds of brinjal were sown in different pot, treatment-wise. The PGPR culture was collected from the IESD, BHU. Initially, the blocks of soil were broken down, homogenized, sieved and filled in the pots (diameter of pots, 7 cm and height 15 cm). Total 70 pots (5 replicates for each treatment × 14 treatments) were prepared and irrigated with tap water to maintain the moisture in the soil to support plant growth. All pots were placed in an experimental area that was covered with nylon net at the top and sides to prevent litter blown from external sources or the bird herbivory. After few days of germination, thinning was done in each pot (reduced up to 1 in each pot) for further experiment. Three bacterial strains (*Paenibacillus polymyxa, Pseudomonas putida* and *Azospirillim brasilense*) and one fungal species (*Trichoderma* sp.) were selected for consortia development. The treatments were: first four mono-inoculants (T1 to T4); *Paenibacillus polymyxa* (T1), *Pseudomonas putida* (T2), *Trichoderma*sp. (T3), *Azospirillum brasilense* (T4), next six bi-inoculants (T5 to T10); *Paenibacillus polymyxa + Pseudomonas putida* (T5), *Paenibacillus polymyxa + Trichoderma* sp. (T6), *Paenibacillus polymyxa + Azospirillum brasilense* (T7), *Pseudomonas putida + Trichoderma* sp.(T8), *Pseudomonas putida + Azospirillum brasilense* (T9), *Trichoderma* sp.+ *Azospirillum brasilense* (T10), next two are tri-inoculants (T11 and T12); *Paenibacillus polymyxa + Pseudomonas putida + Trichoderma* sp. (T11), *Paenibacillus polymyxa + Pseudomonas putida + Azospirillum brasilens* (T12) and one tetra-inoculants *Paenibacillus polymyxa + Pseudomonas putida + Trichoderma sp.+ Azospirillum brasilense* (T13) and one control (T14). Test plants were monitored continuously after every 25-30 days interval for vegetative analysis (including measurement of plant height, no. of leaves, no. of buds, *etc.*). Determining the above and below-ground biomass of brinjal in different treatments was done after crop harvest. The initial and final soil samples were also drawn for physical and chemical analysis. Soil moisture analysis was determined by oven drying at 105°C. Soil pH was done by a digital pH meter. The water holding capacity of the soil was measured by the brass cup method (Piper, 1966). Soil organic carbon (OC) was estimated by Walkley and Black method (Walkley and Black, 1934) and total nitrogen (TN) by the Micro-Kjeldahl method (Jackson, 1958).

Statistical analysis in the present paper was done using the SPSS package (version 16, U.S.A.) All the values are expressed as mean ± standard error. Mean values were compared by using the least significant difference (LSD) range test procedure at the 5% level of significance. Two-tailed Pearson correlation coefficients between soil properties and crop productivity parameters under different PGPR treatments were calculated to observe the impact of change in soil properties on crop productivity.

RESULTS AND DISCUSSION

The experiment showed differences in physico-chemical properties in soil and crop growth attributes in brinjal due to the application of varied quality of Plant Growth Promoting Rhizobacteria (PGPR). Initial holding capacity (WHC), Organic C (0.4%) and total N (0.03%) were scarce in soil showing degraded conditions. pH was neutral to slightly alkaline (Table 1). In the present experiment, there were increases in fertility status of soil after 3-4 months of the incorporation of PGPR culture. Compared to control; soil moisture increased was a minimum of 6% in T1 treatment and a maximum of 135% in T13 treatment. The water holding capacity was recorded highest in T13 (18.5) followed by T11, T12, T8, T9, T5 and was lowest in T1. Enhancement in WHC was varied from 6% to 89% under different PGPR treatments. The value of pH varied from slightly alkaline to neutral or slightly acidic. The most effective combination applied for soil restoration in this study was tetra inoculants (T13) and after that tri inoculants (T11 and T12). These treatments significantly increased plant growth, biomass and nutrient uptake as compared to mono and bi inoculant treatments and control. Soil moisture and WHC were showed little increase in mono inoculant treatments (T1 to T4) and maximum upto 90% in tetra inoculant treatment (T13). *Pseudomonas* sp. is reported to improve soil texture by increasing the water holding capacity of the soil (Tewari and Arora, 2014; Naseem and Bano, 2014). According to Grover *et al.,* (2011), *Azospirillum* sp. is also responsible for increased water circulation between soil and plants. Zhang *et al.* (2020) also reported that the strains of *Pseudomonas* and *Bacillus sp.* are efficient in eliminating drought stress and promoting plant growth. Synergistic effects of bacterial strains used in tetra or tri inoculants in this study (like in T13, T11 and T12) had the maximum values for WHC, soil moisture, OC, TN and plant biomass. Single and bi inoculants showed minimum improvement as promotion of only a few metabolisms in the plant that could not produce synergistic effect. The presence of microbial culture in soil enhances the biodegradation of organic matter, improving

Table 1: Changes in selected soil physico-chemical properties and vegetative characteristics of test crop (brinjal) after application of different PGPR strains in form of single (T1- T4), bi-(T5-T10), tri-(T11-T12) and tetra-(T13) inoculants.

Treatments	SM	WHC	pH	Organic C	Total N	Height	Leaves	Height	Leaves	Height	Leaves
Initial soil properties	6.9±0.24	9.2±1.8	7.6±0.03	0.41±0.02	0.04±0.001	25 DAT		50 DAT		80 DAT	
Paenibacillus polymyxa (T1)	7.8±0.06	10.4±0.15	7.7±0.04	0.63±0.007	0.06±0.0008	1±0.08	5±0.35	9.4±0.08	8±0.6	10.5±0.11	8±0.36
Pseudomonas putida (T2)	9.4±0.13	11.2±0.09	7.4±0.03	0.87±0.014	0.09±0.0007	1.3±0.07	3±0.25	10.0±0.12	9±0.56	12.1±0.06	10±0.25
Trichoderma sp. (T3)	8.6±0.04	11.1±0.19	7.5±0.04	0.72±0.006	0.07±0.0005	1.8±0.05	3±0.25	10.2±0.08	8±0.35	12.2±0.06	9±0.70
Azospirillum brasilense (T4)	8.2±0.08	10.8±0.34	7.6±0.02	0.62±0.006	0.06±0.0008	0.9±0.02	5±0.35	13.5±0.13	9±0.25	15.5±0.11	10±0.25
Paenibacillus polymyxa+ Pseudomonas putida (T5)	13.1±0.06	13.4±0.10	7.3±0.03	1.02±0.01	0.12±0.010	2.0±0.02	2±0.25	13.4±0.08	8±.035	15.4±0.06	10±0.43
Paenibacillus polymyxa + Trichoderma sp. (T6)	12.8±0.18	13.2±0.27	7.3±0.02	1.00±0.014	0.11±0.004	2.5±0.04	2±0.26	11.9±0.14	8±0.25	13.9±0.08	9±0.66
Paenibacillus polymyxa + Azospirillum brasilense (T7)	10.2±0.07	11.8±0.10	7.3±0.02	0.92±0.007	0.09±0.0009	2.5±0.04	3±0.25	11.5±0.08	8±0.35	13.5±0.07	9±0.56
Pseudomonas putida + Trichoderma sp. (T8)	14.2±0.09	14.4±0.14	7.1±0.06	1.19±0.003	0.13±0.0017	2.3±0.07	5±0.25	14.8±0.09	10±0.26	16.5±0.06	10±0.25
Pseudomonas putida + Azospirillum brasilense (T9)	14.0±0.22	14.2±0.17	7.2±0.03	1.17±0.008	0.13±0.001	1.4±0.02	3±0.35	12.0±0.08	9±0.36	14.2±0.07	9±0.35
Trichoderma sp. + Azospirillum brasilense (T10)	12.4±0.19	12.2±0.17	7.3±0.02	0.97±0.007	0.12±0.005	2.3±0.09	5±0.36	12.4±0.05	8±0.25	14.4±0.04	9±0.66
Paenibacillus polymyxa + Pseudomonas putida + Trichoderma sp. (T11)	16.3±0.16	16.6±0.15	7.0±0.04	1.27±0.017	0.13±0.001	1.2±0.05	3±0.25	16.9±0.04	11±0.43	18.1±0.08	11±0.43
Paenibacillus polymyxa + Pseudomonas putida + Azospirillum brasilens (T12)	14.5±0.17	15.5±0.18	7.1±0.03	1.23±0.008	0.14±0.005	2.1±0.06	2±0.25	16.1±0.04	11±0.26	17.9±0.08	11±0.25
Paenibacillus polymyxa + Pseudomonas putida + Trichoderma sp.+ Azospirillum brasilense (T13)	17.3±0.18	18.5±0.20	6.8±0.02	1.30±0.012	0.14±0.001	2.5±0.04	5±0.35	17.9±0.09	12±0.56	19.4±0.08	13±0.35
Control (T14)	7.36±0.88	9.8±0.20	7.6±0.06	0.45±0.005	0.042±0.001	1±0.04	3±0.36	9.0±0.05	7±0.35	10±0.05	8±0.36
LSD	0.49	1.70	0.13	0.034	0.009	0.22	0.31	0.27	1.07	1.31	1.60

Abbreviations: SM= Soil moisture; WHC= Water holding capacity; C= Carbon; N= Nitrogen.

Vegetative analysis was done at 25, 50 and 80 days after transplanting (DAT). Heights are in cm and leaves are no. per plant per treatment. Values are means of replication (±SE).

the pH, improving the nutrient availability for plants and also increasing the fertility of the soil (Adeleke *et al.,* 2017).

The increase in organic carbon (C) and total nitrogen (N) content in soil was evident after the treatment with PGPR culture (Table 1). This increase was maximum in T13 followed by T11> T12> T8> T9> T5> T6> T10> T2> T3> T4> T1 treatment and control. T13 showed an increase in the OC content of soil (1.3) which is about 189% more than the control (0.45), facilitating the accumulation of a large amount of organic matter promoting the significant growth of the plant. The values of total N content in soil was also found maximum in T13 treatment (0.14) followed by T12, T11, T8 and T9 (0.13 in each)> T10 and T5 (0.12)>T6 (0.11)> T7 and T2 (0.09)> T3(0.07) and T1 and T4 (0.06) treatments in comparison to control (0.042). T13 (tetra inoculants) treatment showed 2.8 times greater OC and 3.3 times higher total N as compared to control (Table 1). Improved nutrient acquisition in T13 (tetra-inoculant) and then T11 and T12 (tri-inoculant) treatments was due to increased nutrient uptake of N from nitrogen-fixing bacteria, uptake of P from phosphate mineral solubilizing bacteriaand uptake of iron from siderophore producing bacteria (*Pseudomoas putida* and *Trichoderma sp.*) and suppressing plant diseases (Verma *et al.,* 2016; Jaiswal *et al.*, 2019). It was also reported by Sani *et al.* (2020) that *Trichoderma* sp. improved soil fertility and promoted the growth of rhizosphere microbes, which eventually led to higher tomato yields and increases in antioxidants and minerals. In the present study, the T13 treatment and other treatments were these two species *Pseudomoas putida* and *Trichoderma* sp. showed benefits.

Vegetative growth of test crop (brinjal) also showed improvement under different PGPR treatments. The pattern was more distinct as crops reach maturity (Table 1). The maximum number of leaves has been recorded at 80 days after transplanting DAT in T13 (13 leaves) treatment, followed by T11=T12 (11 leaves)> T2=T4=T5=T8 (10 leaves) > T3=T6=T7=T9=T10 (9 leaves) and minimum in T1 (8 leaves) and control (7 leaves). In all treatments, T13 showed increased shoot growth and the increase was more pronounced at 80 DAT. T13 treatment showed about 2 times more plant leaves and 1.6 times greater shoot height as compared to control.

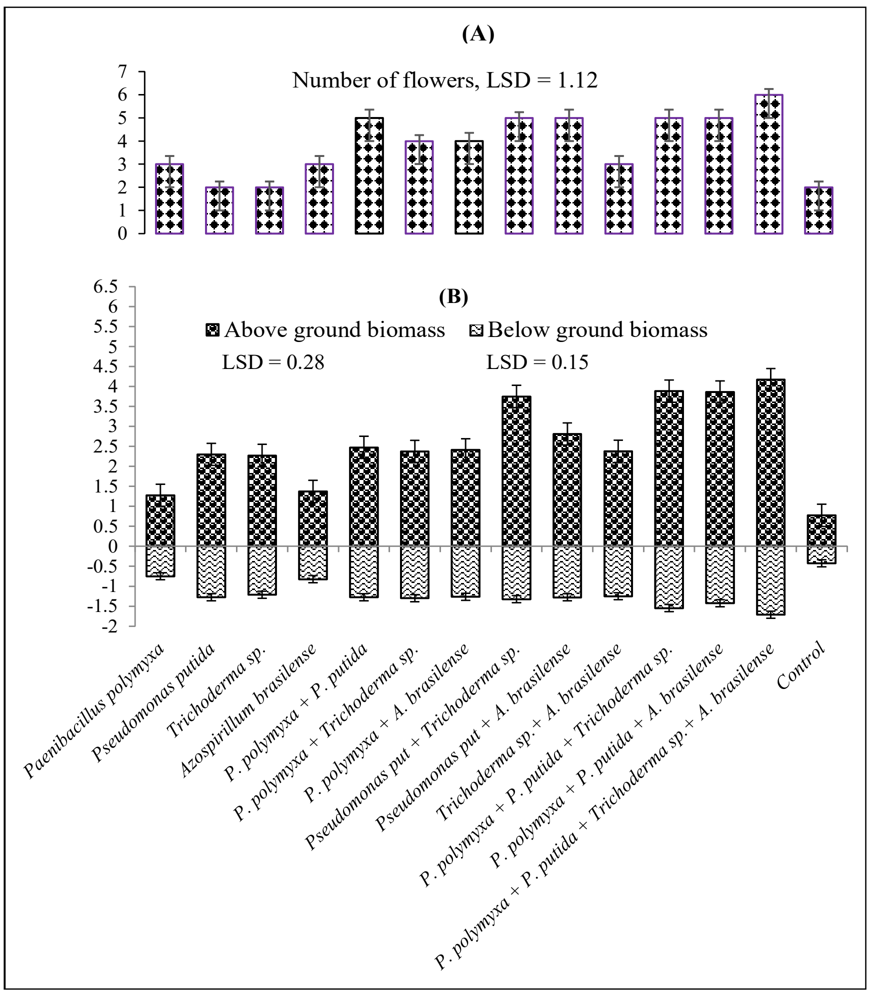

Fig 1: (A) Variations in number of flowers (per plant/pot) (B) above-ground biomass (g/plant/pot) and below-ground biomass g/plant/pot) of test crop under different PGPR treatments. Values are means of ±SE.

Table 2: Pearson's correlation coefficient analysis showing relationship among soil quality indicators and crop productivity parameters.

	Plant height (cm)	Number of leaves (No./plant)	Number of flowers (No./plant)	Above-ground biomass (g/plant)	Below-ground biomass (g/plant)
Soil quality indicators					
Soil moisture (%)	0.87**	0.58*	0.76**	0.90**	0.82**
Water holding capacity (%)	0.76**	0.50*	0.70**	0.75**	0.76**
Soil organic carbon (%)	0.83**	0.52*	0.74**	0.93**	0.85**
Total nitrogen (%)	0.81**	0.51*	0.74**	0.87**	0.82**

All values are significant at the 0.01(**) and 0.5 (*) levels. In the case of plant height, the number of leaves and flowers, final 80 DAT are considered.

Application of mono, bi, tri and tetra PGPR inoculant showed variation in above-ground and below-ground biomass and also in the number of flowers per plant per pot (Fig 1). Maximum above ground (4 g) and below-ground biomass (1.7 g) were recorded in the T13 treatment. However, the treatments such as T10, T6, T7, T3, T4 and T1 (2.4, 2.3, 2.4, 2.3, 1.4 and 1.3 g, respectively) showed comparatively lesser enhancement in biomass comparison to control. Maximum 6 flowers were produced in T13 treatment whereas only 2 flowers per plant were reported in T2, T3 and control. Various studies revealed that *Pseudomonas putida* have a multifunctional role in plant growth promotion and development. It was reported that this species help in biocontrol and produce secondary metabolites and also used for production of IAA, ammonia and increased nutrient uptake (Verma *et al.,* 2014). *Pseudomonas sp.* is also reported to regulate protein synthesis, seed germination, siderophore production and phyto-beneficial traits (Zhang *et al.,* 2019, Dhawi, 2020). Previous studies have also revealed that combined inoculation of *A. Brailense* with *Pseudomonas* sp. and *P. polymyxa*had significantly increased grain yield and dry matter content, N and P uptake (Silva *et al.,* 2015). Benmati *et al.* (2020) investigated the effects of *Azospirillum brasilense*, *Bacillus* sp. and *Frankia* Ccl3 on durum wheat under water deficit conditions also. Their studies confirm the significant abilities of PGPR under water stress conditions for maintaining growth and plant survival. *Azospirillum* sp. is reported to improve plant growth by N fixation and production of IAA, cytokinin and gibberellins (Steenhoudt and Vanderleyden, 2000). Tetra and tri inoculants involving *P. putida* and *Azospirillum* sp. showed the highest values in terms of biomass production and vegetative characteristics compared to other treatments and control. It was reported that *Pseudomonas* and *Trichooderma* sp. showed inhibition of soil-borne phytopathogens (*Fusarium and Rhizoctonia*) by HCN, siderophore production (Gupta and Gopal, 2008) and lytic enzymes (Verma *et al.,* 2014).

Different soil fertility and productivity parameters of the brinjal crop were correlated and assessed under different treatments (Table 2). All soil quality indicators (soil moisture, water holding capacity, organic C and total N) were positively correlated with crop vegetative growth. Greater degrees of correlation were observed in the case of the plant above ground biomass and below-ground biomass with soil organic C (0.93 and 0.85) and total N (0.93 and 0.82).The overall performance of inoculated treatments revealed that seed treatment of Brinjal with tetra and tri inoculants, each bestowed with specific functions such as nitrogen fixer, phosphate solubilizers, antagonist, *etc*, could give significantly better performance in all respect of plant growth, yield, biomass and nutrients uptake as compared to individual treatments.

CONCLUSION

It can be concluded that the most effective treatment to enhance plant growth, biomass and nutrient uptake were tetra (T13, *Paenibacillus polymyxa* + *Pseudomonas putida* + *Trichoderma sp.*+ *Azospirillum brasilense*) and tri inoculants (T12, *Paenibacillus polymyxa* + *Pseudomonas putida* + *Azospirillum brasilen* and T11, *Paenibacillus polymyxa* + *Pseudomonas putida* + *Trichoderma* sp.). Significant increments were not only seen in plant growth but also in the soil physico-chemical properties. The findings thereby confirm that application of microbial consortia (PGPR) in three or more combinations may produce more profound effects in terms of restoration of soil fertility and crop productivity and yield in Vindhyan dry tropical soil.

Conflict of interest: None.

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