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Original Article

MAIZE RHIZOSPHERE MICROBIAL POPULATION IN SOILS OF JHARKHAND

ASHA MISHRA, SHASHWATI GHOSH SACHAN, *DEV MANI PANDEY

Department of Bio-Engineering, Birla Institute of Technology, Mesra, Ranchi, Jharkhand 835215
Email: dmpandey@bitmesra.ac.in

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ABSTRACT

Objective: The main objective of the present investigation is to isolate plant growth promoting rhizobacteria (PGPR) strains from three different zone of rhizosphere and arhizosphere soil, observing bacterial density and exploring rhizosphere effect on the bacterial community.

Methods: Maize rhizosphere soils were collected from twelve different locations of Jharkhand. Various microbial populations (like *Pseudomonas* spp., *Azotobacter* spp., phosphate solubilizing bacterial spp. and *Azospirillum* spp.) were isolated from the different variety of maize rhizosphere. These strains were obtained through pour plate technique by using PGPR specific medium. To explore rhizosphere effects on the bacterial community, some physicochemical properties of arhizosphere soil and rhizosphere soil were measured and the soil bacterial community which helps in plant growth through direct and indirect mechanisms called plant growth promoting rhizobacteria (PGPR) detected by morphological and biochemical characterization. PGPR was purified and subcultured in their respective medium and stored at 4^0C to maintain throughout the experiment.

Results: Total 65 isolates of *Pseudomonas* strain, 34 isolates of *Azotobacter* strain, 49 isolates of phosphate solubilizing bacterial strain and 34 isolates of *Azospirillum* strain were isolated from maize rhizosphere. The pH of rhizosphere soil was lower than arhizosphere soil and rhizosphere soils contain more moisture than arhizosphere soil. Highest moisture % was found in Deoghar (26.90%) followed by Dhurwa (21.73%) and Tamar (21.21%) rhizosphere soils. Microbial population was highest in rhizoplane zone. Organic carbon, available nitrogen, phosphorus, potassium and sulphur were found more in rhizosphere soil than arhizosphere soil. High quantity of organic matter (1.52%), available nitrogen (272.83 kg ha^{-1}), available phosphorus (73.64 kg ha^{-1}), potassium (317.42 kg ha^{-1}) and sulphur (66.60 kg ha^{-1}) were present in rhizosphere soil.

Conclusion: This study reveals that rhizosphere soil characterized by greater microbial population and their activity which enhance soil fertility through increasing moisture, pH and available nutrients in soil to promote the growth of plants.

Keywords: Arhizosphere, Azospirillum, PGPR, Phosphate solubilizing bacteria, Rhizosphere

INTRODUCTION

Rhizosphere soils are root associated soil which has rich microbial population and it is high microbial activity zone. The term "Rhizosphere" was first given by Hiltner [1]. Bacterial population which helps in plant growth is called plant growth promoting rhizobacteria (PGPR). The term "PGPR" was first proposed by Kolepper and Schroth [2] to describe effect of PGPR on plant growth after inoculation to seed. Bacteria have a very broad pH range where they can survive. The rhizosphere pH is usually lower than arhizosphere soil in 1-2 units [3, 4]. PGPR are the most numerous organisms in the soil averaging between 10^{10} to 10^{12} organisms per gram of rhizosphere soil. More than fifteen percent of the root surface area is covered with rhizosphere specific microorganism. Microbial root colonization covers 15-40 % of the total plant root surface [4, 5]. In comparison with arhizosphere soil, pH is lower and organic carbon content and organic matter is usually high in rhizosphere soil. Soil microorganisms like *Pseudomonas* spp., *Azotobacter* spp., phosphate solubilizing bacteria and *Azospirillum* spp. have enhancing capacity by promoting the secondary growth of roots. *Pseudomonas* spp., *Azotobacter* spp., phosphate solubilizing bacteria and *Azospirillum* spp. have been isolated from the arhizosphere soil, rhizosphere soil and roots of a variety of plants including cereals and grasses. Inoculation with indigenous PGPR strains is an important procedure to enhance crop yields and soil fertility. PGPR isolates could be inoculated into crops by either soil treatments, seed treatments or foliar treatments. Rhizosphere concentrations were enriched in nutrients relative to arhizosphere soil [6, 7].

PGPR isolates like *Pseudomonas* spp., *Azotobacter* spp., phosphate solubilizing bacteria and *Azospirillum* spp. are commonly found in soil and associated with plant roots namely rice, maize, wheat, legumes, potato, tomato, soybean etc. Rhizosphere colonization by PGPR strains has stimulated the growth of the different variety of plant species. The success of interaction between plant and PGPR strains depends on the survival and persistence of these bacteria in soil and the effective colonization of the rhizosphere. Soil minerals, moisture, pH, plant varieties, amino acids and root exudates are the survival properties which may contribute to increase the population of PGPR strains, rhizosphere colonization and the initiation of mutualistic interaction by PGPR strains.

Day by day our population is increasing and our agricultural land is decreasing due to the concrete network. There is need for lots of food to fulfil nutritional requirements of growing population and huge land for production of plenty of food crops. Unfortunately we have limited land and we had great challenges to solve this problem. Crops need several nutrients to reach their maximum potential yield. Initially, soil fertility was increased by use of chemical fertilizer but frequent use of chemical fertilizer decreased crop yields and soil fertility and give an adverse effect on the environment including soil and water deterioration and contamination. There is another problem associated with chemical fertilizer is its increasing cost, wide gap between supply and demand. That is why we search another option to combat this problem and we found one and only safe option is microbial consortia which contain beneficial soil microorganisms.

MATERIALS AND METHODS

Sample collections site

Maize plants with rhizosphere soil are collected from selected district of Jharkhand like Ranchi (BAU Kanke, HEC Dhurwa, Bundu and Tamar), Ramgarh (Patratu), West Singhbhum (Chandil), East Singhbhum (Darisahi), Simdega, Gumla, Loherdaga, Daltonganj (Chianki) and Deoghar (table 1). Rhizosphere zone is divided into three zone (i) Rhizosphere zone (R zone), (ii) Rhizoplane zone (Rp zone) and Endorhizosphere zone (ER zone). Rhizospheric soil (0-15 cm) and plant sample were collected from above locations and brought to laboratory. Bacteria were isolated from different rhizotic zones of rhizosphere.

Table 1: PGPR isolation from soil collected from different location of Jharkhand

S. No.	Location	Maize variety
1	BAU Kanke	SUWAN composite
2	Dhurwa	Kanchan 25
3	Bundu	Kanchan 25
4	Tamar	BM1, P11, PR1/A
5	Patratu	+self 1025
6	Chandil	SUWAN composite
7	Darisahi	SUWAN composite
8	Simdega	SPPM
9	Gumla	SUWAN composite
10	Loherdaga	SUWAN composite
11	Chianki	+self 1025
12	Deoghar	SUWAN composite

Analysis of physico-chemical parameter of the arhizosphere and rhizosphere soil

Moisture and pH: Moisture content was determined as per method described earlier [8]. A weighed quantity of the soil was heated at 105 ^{0}C for 4-5 h and the loss of weight was expressed as moisture percentage of the soil. The pH was measured with the help of pH meter to maintain the soil-water ratio of 1:2.5 as described previously [9].

Nutrient analysis (organic carbon, available N, P, K and S) of arhizosphere and rhizosphere soil: Total organic carbon was determined by rapid titration method described previously [10]. Available Nitrogen was determined by alkaline potassium permangnate method [11]. Available Phosphorous was determined by Olsen's method [12]. Soil was extracted by neutral ammonium acetate solution and exchangeable potassium was estimated with the help of flame photometer [9]. For estimation of available sulphur, sulphur was extracted with 0.15% calcium chloride solution from soil and determined by the monocalcium phosphate-turbidimetric method [13, 14].

Isolation of PGPR strains

Different PGPR isolates were isolated by using selective or non selective media. 10 g soil from the rhizosphere of maize plant from selected site were taken and prepared 10 fold dilution series up to 10^{-6} by serial dilution method. One ml aliquots from 10^{-6} dilutions were poured on plates containing respective media. The aliquots were spread on the plate by clockwise and anticlockwise movement under aseptic condition. PGPR were isolated from the arhizospheric soil, rhizoplane and endorhizospheric soil of maize crop fields from different location of Jharkhand during 2010 and 2012 in different field conditions. The population of *Azospirillum* spp. in soil as well as root were enumerated by employing MPN method. This method based upon the pattern of positive and negative growth of *Azospirillum* in the tube inoculated with a consecutive series of dilutions of the soil/root samples. Based on the number of positive tube the population estimated with the help of MPN table [15, 16]. The rhizosphere effect on microbial population can be measured by comparing the microbial population (cfu) between the rhizosphere soil and arhizosphere soil for which the R/S ratio was employed [17].

RESULT AND DISCUSSION

Rhizosphere effects on physico-chemical properties of soil

Rhizosphere soil is wetter than arhizosphere soil. Highest % moisture was recorded in Deoghar rhizosphere soil (26.90%) followed by Dhurwa (21.73%) and Tamar (21.21%) due to waste products and secretions. Constant moisture was recorded B. A. U. Kanke and Patratu rhizosphere soil (fig. 1). These findings are supported by previous study [18]. Among the twelve places of Jharkhand, soil pH varied between 4.5 to 9.7 indicating that PGPR could grow usually in neutral soil (seven places out of twelve), somewhere in acidic soli (four places out of twelve places) and exceptionally in alkaline soil because bacteria can grow and survive on very broad pH range (one out of twelve places). These findings have earlier been reported previously by several workers [3, 4]. Rhizosphere soil pH was lower than arhizosphere soil except Chianki, B. A. U. Kanke, Tamar, Patratu, Simdega and Loherdaga rhizosphre soil have neutral pH that indicates sufficient growth of PGPR in this soil. Rhizosphere soil from Dhurwa, Bundu, Chandil, Darisahi, Gumla and Deoghar have acidic pH that might be due to release of organic acids, amino acids, sugars and proteins. Chianki rhizosphere soil shows alkaline pH. PGPR was grown on acidic, neutral and alkaline pH because bacteria have a very broad pH spectrum where they can survive. Results observed during present investigation may be supported by the work carried out previously [18, 19]. The rhizosphere pH was generally lower than the arhizosphere soil like Dhurwa arhizosphere soil pH 5.2 and rhizosphere soil pH 4.5 followed by Bundu arhizosphere soil pH 6.9 and rhizosphere pH 5.5, Chandil arhizosphere soil pH 6.2 and rhizosphere pH 5.4, Gumla arhizosphere soil pH 6.2 and rhizosphere pH 5.3 and Lohardaga arhizosphere soil pH 7.0 and rhizosphere pH 6.0. The pH values were not significantly different between arhizosphere and rhizosphere soil of Tamar, Patratu, Chandil, Darisahi, Simdega and Deoghar. Obtained findings are in agreement with the results described in previous reports [3, 20].

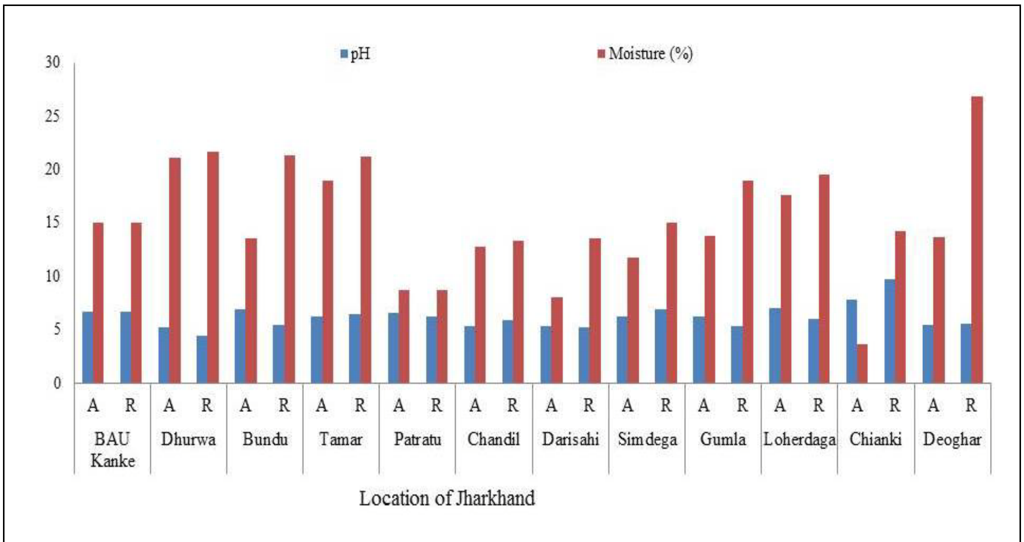

Fig. 1: Difference in moisture (%) and pH of arhizosphere (abbreviated as "A") and rhizosphere (abbreviated as "R") soil collected from different location of Jharkhand

Organic carbon (Oc) is life line of soil that improves the soil fertility by enhancing a great proportion of nutrients, cation and trace elements that are important for soil growth and it also buffers soil from strong changes in pH [21]. Our result indicated higher organic carbon content, available nitrogen, phosphorus, potassium and sulphur in the rhizosphere soil than arhizosphere soil (fig. 2). Our result is supported by the findings of Liu *et al.* [20]. Highest organic carbon was found in Chianki (1.52%) rhizosphere soil followed by Bundu (0.96%) and Simdega (0.69%). Organic carbon was more in B. A. U. Kanke, Chandil, Darisahi, Simdega, Gumla, Chianki and Deoghar. Organic carbon was constant in Dhurwa rhizosphere and arhizosphere soil. Organic carbon was less in Patratu and Lohardaga rhizosphere and arhizosphere soil. In case of Tamar, organic carbon was more in BM1 and PR1/A maize rhizosphere than arhizosphere soil, and organic carbon less in P11 maize rhizosphere. Available nitrogen was more in eleven places out of twelve places maize rhizosphere except Simdega arhizosphere and rhizosphere soil had constant value of available nitrogen. Highest available nitrogen was found in Gumla rhizosphere soil (291.55 kg ha^{-1}) followed by Deoghar (272.83 kg ha^{-1}) and Lohardaga (263.42 kg ha^{-1}). Available phosphorus concentration was high in rhizosphere soil than arhizosphere of B. A. U. Kanke, Dhurwa, Bundu, Tamar (BM1, PR1/A), Patratu, Chandil, Darisahi, Gumla, Lohardaga, Chianki, Deoghar and was less in Simdega and Tamar (P11) rhizosphere. Highest available phosphate was found in Deoghar rhizosphere soil (73.64 kg ha^{-1}) followed by Chianki (64.69 kg ha^{-1}) and Patratu (63.84 kg ha^{-1}). The findings of high available nitrogen and phosphate in rhizosphere are supported by several researchers [22, 23] and low in this region are supported by Turpault *et al.* [24].

Available potassium was more in maize rhizosphere than arhizosphere of Dhurwa, Tamar, Patratu, Chandil, Darisahi, Gumla, Lohardaga, Deoghar and less in maize rhizosphere with respect to arhizosphere of B. A. U. Kanke, Bundu, Simdega and Chianki. This observation is supported by the findings on sorghum [23] and review of McNear [5]. Highest available sulphur was found in Chandil rhizosphere soil (66.60 kg ha^{-1}) followed by B. A. U. Kanke (54.06 kg ha^{-1}) and Dhurwa (32.75 kg ha^{-1}) (fig. 2). Available sulphur was more in rhizosphere soil than arhizosphere of B. A. U. Kanke, Dhurwa, Chandil and Darisahi. This is in accordance with the results described previously [25, 26]. Available sulphur was less in rhizosphere with respect to arhizosphere of Bundu, Tamar, Patratu, Simdega, Gumla, Lohardaga, Chianki and Deoghar.

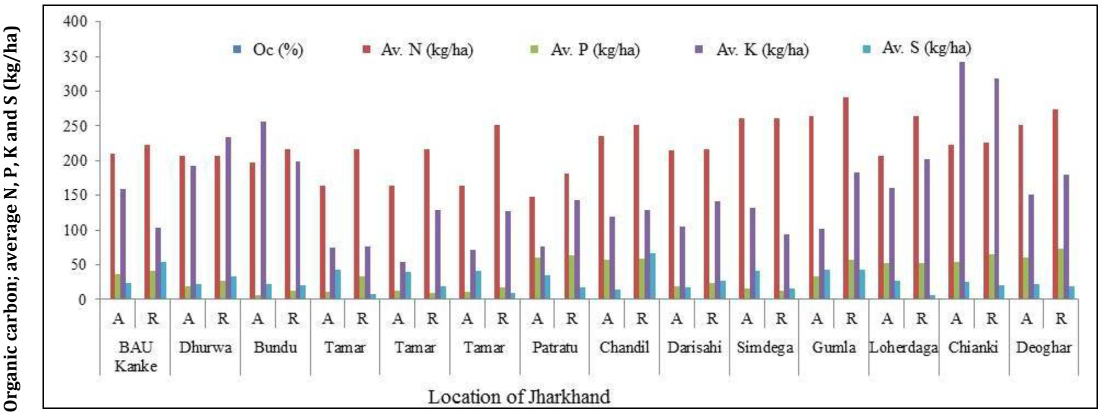

Fig. 2: Differences in available nutrients in arhizosphere (abbreviated as "A") and rhizosphere (abbreviated as "R") soil collected from different location of Jharkhand

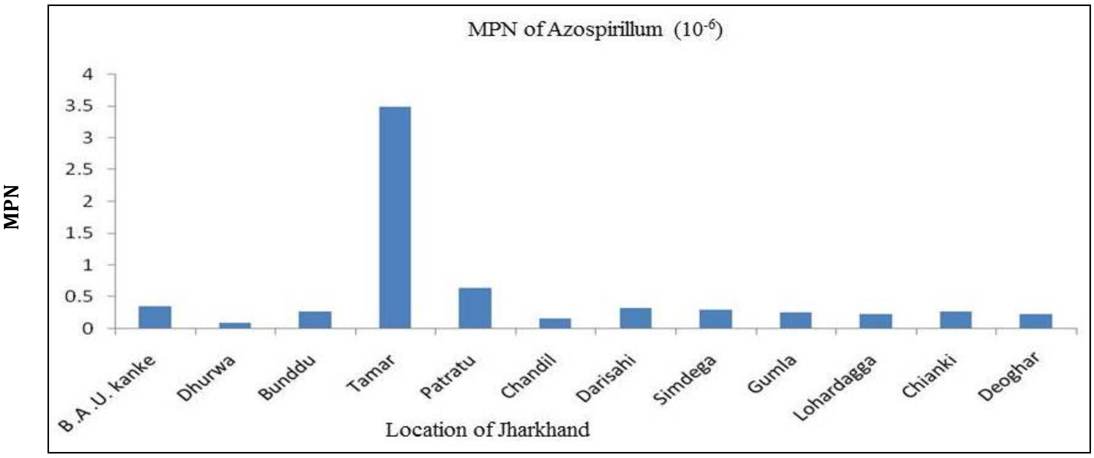

Fig. 3: Population of *Azospirillum* species in maize rhizosphere soil collected from different location of Jharkhand

Rhizosphere effect on microbial population and microbial activity

High level of moisture in the rhizosphere attracts microorganisms than elsewhere in the soil. That is why high population was found in rhizosphere with respect to arhizosphere soil. Identified soil bacterial population was *Pseudomonas* spp., *Azotobacter* spp., Phosphate solubilizing bacteria and *Azospirillum* spp. This work is partially supported by the work of Shi *et al.* [27]. Soil pH affects on rhizosphere microbial population. There were also differences between population at R zone, Rp zone and ER zone. *Pseudomonas* spp. (PSD), *Azotobacter* spp. (AZT), phosphate solubilizing bacteria (PSB) and *Azospirillum* spp (AZS) were isolated from different rhizosphere zone. PGPR population was generally higher in Rp zone. Although there were more PGPR population in Rp zone than R zone but it may be also depends on types of PGPR strain and different places from where soil samples were collected. PSD population were high in Rp zone of eight places out of twelve except Chandil, Chianki and Patratu. PSD population was high in R zone of Chandil and Chianki maize rhizosphere. In case of Patratu maize rhizosphere, population of PSD (144.89 × 10^6cfu), AZT (66.30 ×10^6cfu) and PSB (54.35 ×10^6cfu) were highest in ER zone with comparison to R and Rp zone (table 2). AZT population was highest in R zone of B. A. U. Kanke, Bundu, Darisahi and Simdega maize rhizosphere, highest in Rp zone of Dhurwa, Gumla, Lohardaga and Chianki maize rhizosphere and highest in ER zone of Chandil and Deoghar maize rhizosphere. PSB population was highest in R zone of Bundu and Gumla, highest in Rp zone of Dhurwa, Simdega, Lohardaga and Deoghar, highest in ER zone of B. A. U. Kanke and Darisahi maize rhizosphere except PSB population highest in arhizosphere soil of Chianki. Same highest PSB population (113.30 × 10^6cfu) was found in Chandil maize rhizosphere. In case of Tamar, maize rhizosphere soil sample was taken having three maize varieties BM1, P11 and PR1/A had shown different population density of PSD, AZT and PSB in different zone according to different maize variety rhizosphere. PSD population was highest in Rp zone of BM1 and P11 maize variety rhizosphere but PSD, AZT and PSB population 36.36 × 10^6, 6.42× 10^6 and 10.30× 10^6 was highest in R zone of PR1/R maize rhizosphere, respectively. AZT and PSB population was highest in Rp zone of BM1 variety maize rhizosphere and AZT and PSB population was highest in R zone of P11 variety maize rhizosphere (fig. 3). Tamar maize rhizosphere had highest AZS (3.50× 10^6cfu) population followed by Chandil (0.64× 10^6cfu). Lowest AZS (0.09× 10^6cfu) population was found in Dhurwa followed by population (0.16× 10^6 cfu) in Chandil maize rhizosphere (fig. 3). This work is supported by the work of Bowen [28] and Sylvia [4]. The dense and active microbial population interacts with the roots and within it. The microbial activity in the rhizosphere indicates rhizosphere effect. The rhizosphere effect can be measured by comparing the microbial population (cfu) between the rhizosphere soil (R) and the arhizosphere soil (S), for which the R/S ratio is employed. The R/S ratio was highest in Simdega (43.39) followed by Bundu (31.65) and Lohardaga (30.95). Lowest R/S ratio was found in Chianki followed by Darisahi. Rhizosphere of different places was affected the R/S ratio (fig. 4). This finding is supported by the result described earlier [17].

Table 2: Observation of microbial population in maize arhizosphere (abbreviated as "A") and rhizosphere (abbreviated as "R") soil collected from different location of Jharkhand

S. No.	Location	Sample	PSD cfu 10^6	AZT cfu 10^6	PSB cfu 10^6	Variety
1	B. A. U. Kanke	A	3.45	4.60	5.75	SUWAN composite
		R	41.36	43.46	132.86	
		Rp	101.55	42.13	135.39	
		Er	38.68	24.88	166.06	
2	Dhurwa	A	5.00	2.00	2.00	Kanchan 25
		R	23.33	19.33	10.00	
		Rp	43.33	48.33	16.66	Kanchan 25
3	Bundu	A	0.37	3.75	17.80	
		R	28.1	78.68	71.15	
		Rp	29.22	22.48	8.99	
4	Tamar	A	19.75	7.15	3.87	BM1
		R	14.06	28.60	4.55	
		Rp	65.85	31.51	4.61	
		A	9.044	3.57	3.81	P11
		R	42.42	14.06	10.91	
		Rp	56.48	4.85	0.40	
		A	3.93	1.61	3.87	PR1/A
		R	36.36	6.42	10.30	
		Rp	20.12	0.40	7.51	
5	Patratu	A	11.80	2.17	1.09	+self 1025
		R	47.06	38.04	12.61	
		Rp	7.17	9.78	5.43	
		Er	144.89	66.30	54.35	
6	Chandil	A	1.88	1.81	2.60	SUWAN composite
		R	35.87	10.54	113.30	
		Rp	6.80	2.95	8.61	
		Er	28.32	60.40	113.30	
7	Darisahi	A	39.56	43.24	9.46	SUWAN composite
		R	115.42	85.2	37.83	
		Rp	246.05	82.02	18.86	
		Er	123.03	56.80	40.90	
8	Simdega	A	22.02	1.12	2.24	SPPM
		R	88.09	92.00	38.3	
		Rp	115.00	86.94	115	
		Er	13.34	17.25	0.4	
9	Gumla	A	3.41	1.14	27.99	SUWAN composite
		R	4.76	2.38	29.27	
		Rp	29.75	29.75	5.47	
10	Lohardaga	A	0.40	15.91	21.53	SUWAN composite
		R	1.20	14.35	59.8	
		Rp	89.70	23.92	75.71	
		Er	13.51	21.53	17.1	
11	Chianki	A	34.5	12.43	4.14	+self 1055-6
		R	40.00	11.43	3.77	
		Rp	17.14	17.14	2.99	
		Er	5.71	1.14	2.29	
12	Deoghar	A	2.95	11.37	16.6	SUWAN composite
		R	25.40	10.16	52.83	
		Rp	31.75	44.45	76.2	
		Er	14.73	63.50	67.69	

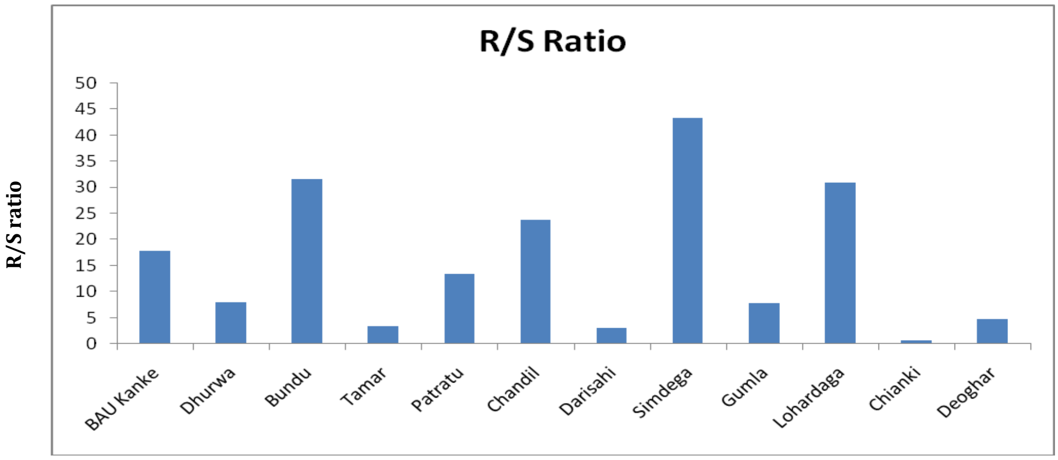

Fig. 4: Rhizosphere effect on R/S ratio in soil collected from different location of Jharkhand

CONCLUSION

In general higher moisture percentage and soil pH was recorded in rhizosphere soil with few exceptions. Critical perusal of data revealed that higher organic carbon, available nitrogen, phosphorus, potassium, sulphur and microbial population was noticed which reflects positive rhizosphere effect. Bacterial population *Pseudomonas* spp., *Azotobacter* spp., phosphate solubilizing bacteria and *Azospirillum* spp. were isolated from maize rhizosphere.

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CONFLICT OF INTERESTS

Declared None

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