Analysis of Bulk and Pigeonpea Rhizosphere Soil in Middle Gangetic Region of Uttar Pradesh

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An investigation was carried out with bulk and pigeonpea rhizosphere soils collected from Middle Gangetic region of Uttar Pradesh (UP). Collected soils were analyzed for physicochemical characteristics, bacterial population and R/S ratio. In the present study, bacterial count of rhizosphere soils were found to range between 250x10^5 to 40x10^5 whereas in bulk soil ranging from 220x10^5 to 14x10^5. The R/S ratio ranging from 5.74 to 1.12, indicating positive rhizospheric effect in collected soil samples. In addition, bacterial population associated with bulk and pigeonpea rhizosphere soils were also evaluated *in vitro* plant growth promoting attributes. In the present investigation, 46%, 16.6%, 75% and 21% of bacterial population associated with rhizosphere soil were found to be phosphate solubilizing, IAA, siderophore and HCN producing respectively whereas in bulk soil 5.5%, 38.8% and 67% of bacterial population were found to be phosphate solubilizing, IAA and siderophore producing respectively. The results suggest that higher percentage of phosphate solubilizers, IAA, siderophore and HCN producers were found in rhizospheric soils of pigeonpea as comparison to bulk soils and may be exploited as biofertilizers or microbial inoculants in the soils of pigeon pea crops growing in the vicinity of middle Gangetic region of Uttar Pradesh.

Keywords: IAA, HCN, Gangetic region and R/S ratio.

Rhizosphere is the region of soil influenced by plant roots that have affluent microbial population and recognized as hot spot for microbial activities. The term “Rhizosphere” was introduced by Hiltner[1]. Soil beneficial bacteria that help in plant growth and development is called plant growth promoting bacteria (PGPB)[2]. The term PGPB was proposed by Bashan and Holguin in 1998 to describe effect of PGPB on growth and yield of plants after inoculation to seed[3]. Bacteria can grow and survive in a broad pH range. The pH of rhizosphere soil is usually less than bulk soil by upto 1-2 units[3, 4]. PGPB are the most abundant microorganisms in the rhizosphere ranging from 10^{10} to 10^{11} organisms per gram of soil[4, 5, 6]. Ample studies have been documented that microbial root colonization covers more than fifteen percent of the total plant root surface[4, 5]. Soil outside rhizosphere and away from plant root system is considered as bulk or arhizosphere soil[6]. Microbial biomass and natural organic compounds are usually lower in bulk soil in comparison to rhizospheric soil[1, 4]. The micronutrient composition of rhizosphere can be very different from that of arhizosphere soil solution due to root exudation, nutrient uptake rhizosphere microbial activity[7].

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Over the past few years, PGPB have gained worldwide importance for sustainable agricultural practices, as a diverse range of soil beneficial bacteria such as *Bacillus, Pseudomonas, Rhizobium, Klebsiella, Azotobacter, Agrobacterium* and *Azospirillum* play vital role in facilitation of plant growth and disease management both under control and field conditions[1, 8, 9]. In addition, use of PGPB in farming practices in order to boost the plant growth via direct and indirect mechanisms are considered an alternative strategy to minimize the use of chemical fertilizers and pesticides as much as feasible[8, 9]. Direct mechanisms involved the facilitation of the uptake of nutrients from the surrounding environments, synthesis of various substances such as phytohormones (auxins, gibberellins and cytokinins) and lowering of ethylene concentration etc[10, 11, 12]. Indirect mechanism of PGPB includes the sequestering of iron from the rhizospheric environment, synthesis of fungicidal compounds and fungal cell wall degrading enzymes, production of antibiotics, competition for root colonization and induced systemic resistance (ISR)[13, 14].

The pigeonpea *(Cajanus cajan)* is a drought tolerant leguminous food crop belongs to family fabaceae, which can be grown on less irrigated land and semi-arid climate conditions. In addition, pigeonpea is principal dryland crop used historically as food, fodder and fuel[15, 16, 17]. Besides being employed as food and fodder this plant even have number of medicinal values and used against fever, dysentery, hepatitis, diabetes and contagious diseases etc[16]. Pigeonpea is the world's most important leguminous crop and India alone account for 80% of world pigeonpea production[17]. Present study investigates the comparison of bacterial population and plant growth promoting activity of bacteria associated with bulk and pigeonpea rhizosphere soil collected in the vicinity of middle Gangetic region of Uttar Pradesh.

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected from bulk and rhizosphere soil of locally cultivated pigeonpea plant during the cropping season from farmer's field covering different areas in the middle Gangetic region of Uttar Pradesh. The soil samples were collected with following standard procedure. Rhizosphere soil sample was taken carefully by uprooting plant and collecting soil volume around the root system. Bulk soil sample was taken by digging soil with the help of augur up to a depth of 0-30 cm at all four corners and at centre of the field[18]. All the corner and centre soils were mixed properly to ensure uniformity in bulk soil sample. Soil samples collected were mixed properly, air dried, crushed to pass through a 2-mm sieve and placed separately in polyethylene bags. All samples collected from the fields were labelled clearly and brought to the laboratory for further analysis.

Soil physicochemical analysis

Test plant rhizosphere and bulk soil samples were analysed for chemical and physical characteristics like soil pH, organic carbon and available nutrients (P and K)[19]. Soil sample properties that determine the availability of plant nutrients and soil fertility were analysed using standard soil test methods described by Lu[19]. Soil pH value was determined by using pH meter in the supernatant solution of 1:5 soil/water ratios (w/v). Soil available organic carbon (OC) was determined using the rapid titration method or wet digestion method of Walkley and Black [20]. Available phosphorus (P) content was extracted by 0.5 M sodium bicarbonate and measured using ascorbic acid-ammonium molybdate method or Olsen's Method[21]. Available potassium (K) content was extracted by 1 mol L^{-1} ammonium acetate and determined by flame photometry[22].

Bacterial count and rhizospheric effect

Bacterial population in the collected soil samples were determined by dilution plate method and expressed in colony forming units (cfu) per gram of soil. Rhizosphere effect is determined by dividing the number of bacterial population per gram of rhizosphere soil by the number of bacterial population per gram of bulk soil[23]. The rhizosphere effect depends on various factors like the nature and amount of root exudates, edaphic factors (soil type and Fertility), and on climatic factors (temperature, rainfall and moisture availability)[24]. The rhizosphere effect greatly decreases with increasing distance from the root, thus R/S ratios determined rhizosphere effects should be carefully measure from different samples.

$$\text{R/S Ratio} = \frac{\text{Number of colonies in the rhizosphere soil}}{\text{Number of colonies in the non- rhizosphere soil}}$$

Evaluation of plant growth promoting attributes

Phosphate solubilization activity on Pikovskaya agar was detected by following the method of Kumar *et al*[25]. Indole-3-acetic acid (IAA) production was estimated by as per method of Gordon and Weber[26] in the presence of L-tryptophan. HCN production was tested by using method of Bakker and Schippers[27]. Siderophore production was determined by chrome azurol sulphonate (CAS) assay by following the method of Schwyn and Neilands[28].

RESULTS AND DISCUSSION

In the present work, bulk and rhizosphere soil samples collected from pigeonpea rhizosphere in the vicinity of middle Gangetic region of Uttar Pradesh were analyzed for different physico-chemical parameters. Among the collected soil samples, pH of soil varied between 6.8 to 8.4 in rhizosphere soils whereas in bulk soil ranging between 6.7 to 8.2 indicating that very slight difference regarding pH was observed in rhizosphere and bulk soils (Fig. 1). Organic carbon (Oc) is vital component in soil that ameliorates the soil fertility by enhancing a good proportion of plant nutrients, trace elements and ions which are essential for plant growth and development and buffers soil against strong pH changes[1]. Differences in available nutrients (K and P) and organic carbon in bulk and rhizosphere soil collected from Middle Gangetic region of UP are illustrated in (Fig. 1 and 2).

Rhizosphere is appropriate niche for microbial populations due to exudation of compounds released by plant roots in root soil interface[1, 25]. Additionally, immense studies revealed that community of microorganisms inhabiting in an explicit environment is specific and dependent solely on the set of ecological and physical factors of that environment[25]. In the present study, bacterial count of rhizosphere soils were found to range between 250x10^5 to 40x10^5 whereas in bulk soil ranging between 220x10^5 to 14x10^5 (Fig. 3). Results suggested that bacterial count of rhizosphere soils were found greater in pigeonpea rhizosphere as compared to bulk soils which might be as a result of volatile compounds released by root cells and soil microorganisms (such as organic acids, sugars, proteins and amino acids). These results are supported by the work of Mishra *et al*[1], Bowen[29], Sylvia[30] and Johri[31] that reported higher population in rhizosphere soils as comparison to bulk soil. Moreover, the dense and active population of microbes interacts with the roots and *root* interior of healthy plants[23, 24]. In addition, microbial abundance and its activity in the root soil interface indicate rhizosphere effect[6]. Rhizosphere effect is determined by dividing the number of bacterial population per gram of rhizosphere soil by the number of bacterial population per gram of bulk soil[23]. In general, the rhizosphere effect greatly decreases with increasing distance from the root[1, 32]. The rhizosphere effect depends on various factors like the nature and amount of root exudates, edaphic factors (soil type and Fertility), and on climatic factors (temperature, rainfall and moisture availability) etc[6]. Rhizosphere of plant species in different places was affected the R/S ratio. In the present study, R/S ratio was highest in Masodha (5.74) followed by Bikapur (5.44) and Faizabad (5.4). Lowest R/S ratio was recorded in Buxa (1.12) followed by Barkaccha (1.16) indicating positive rhizospheric effect in rhizosphere of pigeonpea (Fig. 4). Our results are in close conformation to those of Mishra *et al*[1] and Calvaruso *et al*[32] that

Table 1. Plant growth promoting attributes of bacterial population associated with bulk and pigeonpea rhizosphere soils in the vicinity of middle Gangetic region of UP

Soil sample	Bacterial population (%)			
	IAA production	Phosphate solubiliation	Siderophore production	HCN production
Rhizosphere soil	46	16.6	75	21
Bulk soil	38.8	5.5	67	-

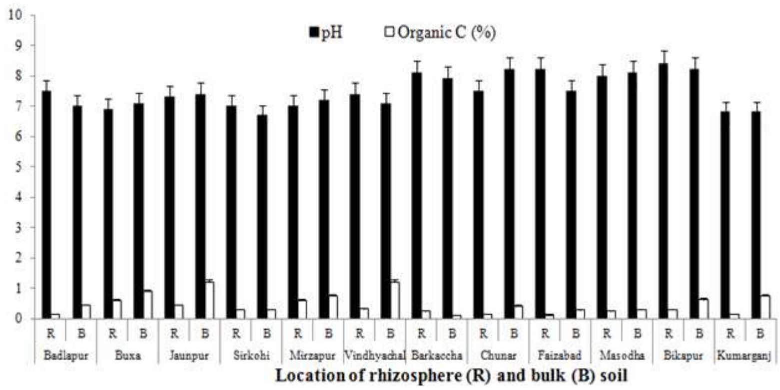

Fig. 1. Differences in pH and available organic C% of bulk and pigeonpea rhizosphere soil collected from Middle Gangetic region of UP

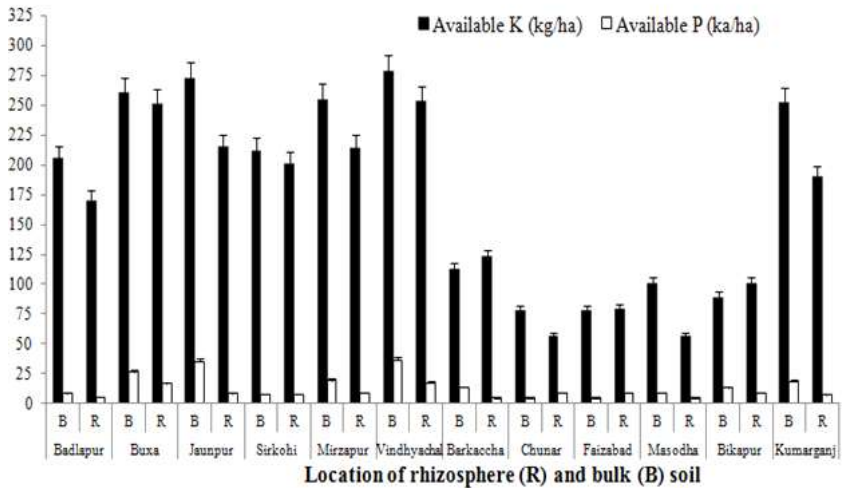

Fig. 2. Differences in available nutrients (K and P) in bulk and rhizosphere soil collected from Middle Gangetic region of UP

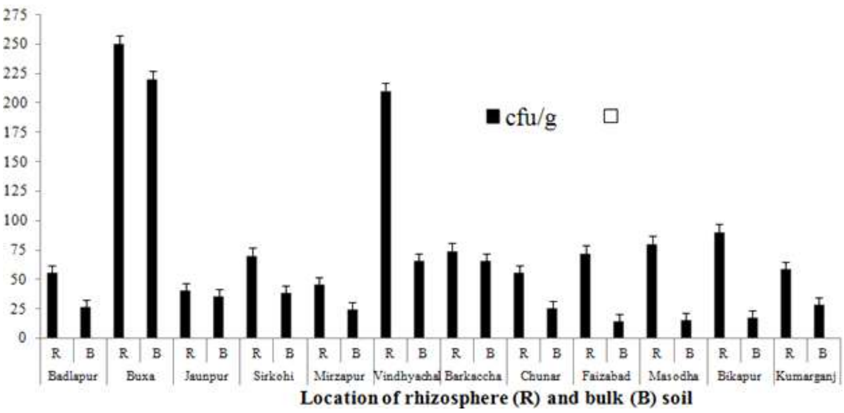

Fig. 3. Bacterial population of bulk and rhizosphere soil collected from Middle Gangetic region of UP

highlights the occurrence of positive rhizospheric effect in maize and Norway Spruce rhizosphere respectively.

Root soil interface is an essential soil ecological environment recognized for various aspects of plant microbe interactions[33]. Interaction of microorganisms and plant in the root soil interface may be beneficial, neutral or deleterious for plant growth[34]. Soil beneficial bacteria that help in plant growth and development is called PGPB[2]. PGPB were defined as soil beneficial bacteria that colonize the roots of plant and promote plant growth via direct and indirect ways[9]. Hence, bacterial population associated with bulk and pigeonpea rhizosphere soils were evaluated *in vitro* plant growth promoting attributes (Table 1). In the present investigation, 46%, 16.6%, 75% and 21% of bacterial population associated with rhizosphere soil were found to be phosphate solubilizing, IAA, siderophore and HCN producing whereas in bulk soil 5.5%, 38.8% and 67% of bacterial population were found to be phosphate solubilizing, IAA and siderophore producing (Table 1). The results suggest that higher percentage of phosphate solubilizers, IAA, siderophore and HCN producers were found in rhizospheric soils of pigeonpea as comparison to bulk soils. Ample studies have been documented that higher concentration of phosphate-solubilizers and IAA producers is usually found in the rhizosphere soils in comparison to bulk soil[2, 9, 35-37]. However, ability of bacteria to produce IAA depends on the culture

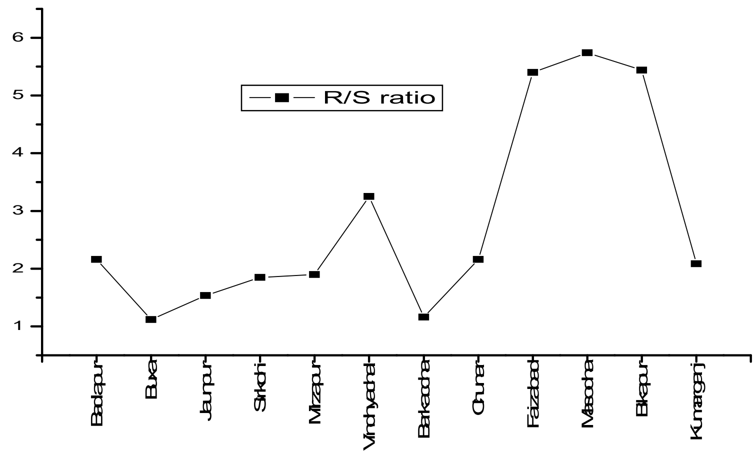

Fig. 4. R/S ratio of different soil samples collected from Middle Gangetic region of UP

condition, growth stage, uptake of microbial IAA by plant and substrate availability[35, 36]. In addition, siderophore and HCN production is another important trait of soil beneficial bacteria that indirectly influences growth of plants by playing essential role in the biological control of plant pathogens[38, 39]. In the present study, none of the isolates isolated from bulk soil produced HCN whereas 21% of bacterial population associated with pigeonpea rhizosphere were found to be HCN producing. It has been reported that bacterial population showing production of HCN plays a pivotal role in biological control of weeds via environmentally compatible mechanism[40].

In conclusion, the present investigation revealed that bacterial population associated with middle Gangetic region of Uttar Pradesh can be used as microbial inoculants for pigeonpea plant and other crops also. However, further studies are needed to confirm the effectiveness of these bacterial populations both under green house and field conditions. Additionally, bacterial populations associated with rhizosphere of pigeonpea crop can be further explored for different aspects of bioprospecting potential.

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