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REGULAR ARTICLE

Studies on soil health and plant growth promoting potential of *Rhizobium* isolates

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ABSTRACT

A comperative study of organically and conventionally managed soils under beans was conducted to ascertain the physicochemical and microbiological characteristics of these soils. Average values of physicochemical and microbiological parameters of the rhizosphere soil samples from the selected districts were compared with the bean rhizosphere soils of the organic farm of Sher-e-Kashmir University of Agricultural Science and Technology of Kashmir India. The results revealed that the soil of the organic farm has significantly higher content of organic carbon (1.04%), available Nitrogen, (298.7 kgha^{-1}) phosphorus (16.72 kgha^{-1}), potassium (296.30 kgha^{-1}), dehydrogenase activity (68.7μg TPF/24 hr g^{-1} soil), total viable bacteria (78.90 × 10^6 cfu g^{-1} soil), fungi (48.73 × 10^3 cfu g^{-1} soil), actinomycete (27.20 × 10^3 cfu g^{-1} soil), phosphate solubilizing bacteria (18.30 × 10^5 cfu g^{-1} soil) and mycorrhizal spores (4.10 spores g^{-1} soil) followed by that of district Kupwara rhizosphere soils with organic carbon (0.97%), available Nitrogen (293.0 kg ha^{-1}), phosphorus (15.81 kg ha^{-1}), potassium (252.3 kg ha^{-1}), dehydrogenase activity (62.7 μg TPF/24 hr g^{-1} soil), total viable bacteria (72.60 × 10^6 cfu g^{-1} soil), fungi (45.76 × 10^3 cfu g^{-1} soil), actinomycete (24.3 × 10^3 cfu g^{-1} soil), phosphate solubilizing bacteria (14.8 × 10^5 cfu g^{-1} soil) and mycorrhizal spores (3.8 spores g^{-1} soil). Rhizobium bacteria were also isolated from the effective nodules of the bean plants grown at different places of various districts in Kashmir valley, India. These isolates after identification were screened for the production of IAA, GA and siderophores. The isolate (*Rhizobium phaseoli* OF) from Organic farm was found most promising by producing 39.20 μl,162 μl, and 24 μl of IAA, GA and siderophor respectively followed by 37.5 μl, 153 μl, and 21 μl of IAA, GA and siderophor respectively from the isolate obtained from rhizosphere soils of Kupwara district. The isolate (*Rhizobium phaseoli* OF) was used in combination with three levels of fertilizer nitrogen (0, 20 and 40 kg ha^{-1}) in a field experiment with beans as experimental crop and five replications by adopting RBD design to study the impact on various plant growth and yield attributing features like number of pods per plant, pod weight and number of nodules. Nitrogen uptake, apparent nitrogen recovery and percent soil nitrogen utilization was also estimated. Maximum number of pods (12 plant^{-1}) was recorded under the treatments T_5 and T_6. Significantly maximum pod weight (5.96 g) and number of nodules (60.45 plant^{-1}) was observed under the treatment T_5. Treatments T_5 and T_6 were at par with respect to nitrogen uptake in grains (63 and 64 kg ha^{-1}), plant biomass (84 kg ha^{-1}) and total N uptake (147 and 148 kg ha^{-1}) by plant. Maximum apparent nitrogen recovery (210) and percent soil nitrogen utilization (46.37) was recorded from the treatment T_5.

Keywords: Rhizobium; Plant growth promoting activities; Nitrogen fertilizer; Soil health

INTRODUCTION

Soil health determines the overall capability of soil ecosystem to support the crop growth and microbial population. Biological and biochemical properties of soil, particularly those involved in energy flow and nutrient cycling, have been found to respond even minimal changes in soil conditions and management, thus furnishing information sensitive to subtle alteration of soil quality (Pascual et al., 2000). Soil enzyme activities can be considered effective indicators of soil quality changes which occur as a result of environmental stresses and management practices. Soil enzyme analysis helps to assess the soil fertility,microbial activity and biochemical cycles of various elements in soils. Dehydrogenase activity is generally used as a measure of any disruption resulted by pesticides, heavy metals and management practices (McCarthy et al., 1994). Assessment of soil

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dehydrogenase enzyme shall be used as a diagnostic tool for better soil ecosystem assessment and amelioration. A large number of bacterial strains have been found to have beneficial impacts on the growth, yield and development of plants. Therefore their application for better plant health management and improving crop production has been the focused area of many studies for past so many years. This category of bacteria has been refered as plant growth promoting bacteria, which include the strains of genera like *Pseudomonas, Azospirillum, Burkholderia, Bacillus, Enterobacter, Rhizobium, Erwinia, Serratia, Alcaligenes, Arthrobacter, Acinetobacter* and *Flavobacterium*. Rhizobial bacteria have been proved to act as promising plant growth promoters. In the recent years the PGPR have attracted a cosmopolitain significance for their applications as a potential tool for sustaining agriculture production and improving soil health (Arshad et al., 2008). The mechanism by which the PGPR affects the plant growth positively can be of two types i.e., direct and indirect. Direct growth promotion can be due to the production of Indole compounds, gibberlic acid, nitrogen fixation, synthesis of ACC deaminase, solubilization of insoluble and unavailable mineral nutrients and mineralization of organic matter, while as indirect mechanism is the prevention of deleterious effect of pathogenic microorganisms. The IAA hormone leads to the plant root system development and subsequently enhances the uptake of nutrients from the soil. Soil bacteria of the family *Rhizobiaceae* which are gram negative, chemolitotrophic or chemo-organotrophic are called Rhizobia (Werner, 1992). This family has an array of bacterial genera; containing *Rhizobium*, *Bradyrhizobium*, *Allorhizobium*, *Mesorhizobium*, *Sinorhizobium* and *Azorhizobium*. From more than a century it has been known that growth of legumes can be promoted by Rhizobia via formation of nitrogen-fixing nodules only but several mechanisms have been proposed by which Rhizobia can directly stimulate the growth of plants including production of phytohormones (Humphry et al., 2007; Patten and Glick, 2000) increased uptake of nutrients (Biswas et al., 2000; Chabot et al., 1996), siderophore production which are compounds having low molecular weight and high affinity for iron (Meyer, 2000).

Beans and peas are popular vegetables that provide a rich source of proteins and carbohydrates. Edible pod are available early in the season before any other vegetables.In addition these play an important role in the crop rotation system by providing the nitrogen to the successive crop with out the added expense of supplimental fertilizer doses. Beans and peas are easy to grow and suffer few diseases and insect problems. Since pulses occupy an important position in the crop profile of Kashmir valley, therefore present study was aimed to assess the soil health of the legume growing soils of Kashmir valley.

MATERIAL AND METHODS

The present experiment was conducted at Regional Research station Wadura campus of SKUAST Kashmir during the years 2011-12 and 2012-13. A total of one hundred ten (110) bean rhizosphere soil samples were collected from five districts of kashmir valley and these were analysed for various physicochemical and microbial characteristics. Rhizobial isolates were also isolated from bean plants grown at different places in these districts. A total of fifty isolates were isolated from the beans roots. Ten plants at each location were uprooted and soil very close to roots was collected, pooled and packed in sterilized containers. The microbial assay was conducted immediately after the collection of the soil samples. The out standing screened *Rhizobium* isolate in combination with three levels of nitrogen (N_0, N_{20}, N_{40} kg^{-1}) was used in a field experiment following RBD design with five replications using french bean as experimental crop.

Physico and biochemical properties of rhizosphere soils of beans

A portion of the collected soil was air dried and processed for the estimation of various soil physicochemical properties. The pH and EC was measured in 1:2.5 soil water suspension with the help of a pH meter (Jackson, 1973), while as the electrical conductivity of the soil water extract was recorded with the help of a wheat stone bridge conductivity meter (Jackson, 1973). Organic carbon was determined by Walkly and Black rapid titration method as given by Pipper (1966), available nitrogen was estimated by Subbiah and Asija method (1956), available phosphorus was determined by Olsen's method as described by Jackson (1973), while as potassium was determined by flame photometer as described by Merwin and Peech (1950). The dehydrogenase avtivity was determined spectrophotometrically by following the method of Klein et al., (1971).

Microbial properties of rhizosphere soils of beans

The total viable bacteria, fungi, actinomycetes and phosphate solubilzing bacteria were enumerated by following dilution plating, agar media, incubation times and temperatures as described by Weaver et al. (1994). The arbuscular mycorrhizal spores were isolated and quantified by the method as described by Gerdemann and Nicolson (1963).

Isolation of Rhizobium from nodules

For isolation of rhizobial strains the bean plants were uprooted and the root system was washed thoroughly with tap water and large sized pink and healthy nodules were separated from the root system by using a sterilized blade, the nodules were then surface disinfected by dipping in 95% ethanol solution for 10 seconds followed by sterilization

by 0.1% mercury chloride for three minutes (Russel et al., 1982). The nodules were then washed by sterilized water several times. The nodules were then crushed by a sterilized glass rod and mixed with 100 µl sterilizes water. A loopful of the suspension was then streaked on YEMA medium and then incubated at 28±2°C for 36 hrs.The single isolated colonies were then picked and restreaked on agar plates, this restreaking process was repeated several times to get pure culture of rhizobia.

Identification of rhizobium strains

The rhizobial isolates were identified on the basis of colony features, cell shape, size, motality, gram staining (Arora, 2003). The isolates were subjected to different biochemical tests like catalase, indole production, methyl red, Vogas prouskauer, citrate utilization (Lowe, 1962), starch hydrolysis, gelatin liquefaction (Arora, 2003).

The isolates were also tested for the fermentation of various sugars including Dextrose, Galactose, Mannose, Citrate, Lactose, Xylose, Fructose, Melibiose, L-arabinose, Glycerol, Mannitol (Krieg and Holt, 1984).

Production of plant growth promoting substance

All the Rhizobial isolates were subjected to qualitative estimation of IAA (Bric et al., 1991) and GA production (Brown and Burlingham, 1968). Luria agar supplemented with 0.06 per cent sodium dodecyl sulphate and one per cent glycerol was prepared and plated. The surface area of the agar medium was divided into squares of 2 cm × 2 cm by marking on the bottom of each plate. The overnight culture of each isolate grown on Luria agar was spotted with sterile tooth pick in each square. The spotted plates were overlaid immediately with sterile disc of Whatman No. 1 filter paper. Plates were incubated until the colonies reached the size of 0.5 to 2.0 mm in diameter. The filter paper discs after incubation period were removed from the plates and treated by soaking in pertidishes containing Salkovaski's reagent (2% of 0.5 m FeCl3 in 35% perchloric acid). The treatment was allowed to proceed until adequate colour was developed. The IAA producing isolates were identified by the formation of characteristic red halo around the colony on filter paper. The paper discs after treatment were viewed under UV light. The spots giving typical green fluorescence were taken as positive for GA production.

The isolates showing IAA and GA production were further examined for quantitative estimation of IAA and GA production as detailed below.

Quantitative estimation of IAA and GA

Extraction

The overnight cultures of the isolates which were positive for the production of IAA and GA in qualitative estimation were inoculated to 50 ml of sterilized Czapeck's solution and incubated at 37°C for seven days in dark. After incubation, the cultures were centrifuged at 6000 rpm for 20 minutes. The supernatant was collected in a conical flask and used for estimation of IAA and GA.

Estimation of IAA (Gordon and Paleg, 1957)

Twenty five ml of the supernatant was collected and the pH was adjusted to 2.8 using 1N HCl in a 100 ml conical flask. Equal volume of diethyl ether was added to it and incubated in dark for four hours. Extraction of IAA was done at 40°C in a separating funnel using diethyl either. The organic phase was discarded and the solvent phase was pooled and evaporated to dryness. To the dried material, two ml of methanol was added and the IAA present in the methanol extract was determined. To 0.5 ml of methanol extract, 1.5 ml of distilled water and four ml Sapler's reagent (1 ml of 0.5 M FeCl3 in 50 ml of 35% perchloric acid) were added and incubated in dark for one hour. The intensity of pink colour developed was read at 535 nm in a UV-VIS spectrophotometer. From a standard curve prepared with known concentrations of IAA, the quantity of IAA in the culture filtrate was determined and expressed as g per litre of broth medium.

Estimation of GA (Paleg, 1965)

Twenty five ml of the culture filtrate was taken in a test tube to which two ml of zinc acetate was added. After two minutes, two ml of potassium ferrocyanide was added and centrifuged at 1000 rpm for 15 minutes. To five ml of this supernatant was added five ml of 30 per cent HCl and incubated at 20°C for 75 minutes. The blank sample was treated with five per cent HCl and the absorbance of the sample as well as blank was measured at 254 nm in a UV-visible spectrophotometer. The amount of GA present in the extract was calculated from the standard curve and expressed as g per litre of the medium. The standard curves of IAA and GA were prepared by using graded concentrations of IAA and GA.

Qualitative test for siderophore production

Production of siderophore by rhizobial isolates was determined by plate assay. Chrome Azurol S blue agar medium (CAS) was used to detect siderophore production (Schwyn and Neilands, 1987). For preparing one litre of CAS medium, 60.5 mg Chrome Azurol S (CAS) (HIMEDIA) was dissolved in 50 ml water and mixed with 10 ml iron (III) solution (1 mM $FeCl_3.6H_2O$ in 10 mM HCl). While stirring, this solution was slowly added to 72.9 mg hexadecyl trimethyl ammonium bromide (HDTMA) dissolved in 40 ml water. The resultant dark blue liquid was autoclaved. To 100 ml of Ashby's broth, 30.2 g of PIPES, 18 g of Difco Agar and 750 ml double distilled water were added. The pH of the medium was adjusted to 6.8 by the

addition of NaOH solution (w/v) and autoclaved. After cooling to 50°C, the dye solution was added along the glass wall with gentle agitation to achieve mixing without formation of foam. The medium was poured into sterile petriplates. The plates were stored in a refrigerator (4°C) for 24 h before use. The overnight grown bacteria were spotted on CAS plates and incubated at 30°C for 24 h. The cultures showing yellow to orange coloured zone around the colonies were taken as positive for siderophore production.

Quantitative estimation of siderophore production:

Production of siderophore by the rhizobial isolates was estimated by the method described by Reeves *et al.* (1983). One hundred ml of Ashby's medium broth was inoculated with one per cent v/v standard inoculum (10^9 cfu/ml) and incubated at 28±2^{0}C for seven days. The culture was centrifuged at 12000 rpm for 30 min. Twenty ml of culture filtrate was extracted twice with equal amount of ethyl acetate after adjusting the pH to 2.0 with 0.1 N HCl. The ethyl acetate top phase was pooled, air dried and dissolved in 5 ml of distilled water. Five ml of Hathway reagent (1 ml of 0.1 M $FeCl_3$ and 1 ml of 0.1 N HCl to 100 ml of distilled water followed by addition of 1 ml of 0.1 M potassium ferricyanide) was added to the assay solution and allowed to stand for the colour to develop. To estimate catechol type of siderophore, the absorbance was read at 700 nm with 2, 3- dihydroxy benzoic acid as standard. The quantity of siderophore synthesized was expressed as g per ml of culture filtrate.

Dehydrogenase activity

The dehydrogenase activity was determined by placing 1 gm air dried soil sample in screw capped test tube, 0.2 and 0.5 ml of 3% 2,3,5 tetrazolium chloride and 1% glucose solution was added to the sample before incubation at 28±0.5 ^{0}C for 24 hours,after incubation 10 ml of methanol were added followed by vigrous shaking and then allowed to stand for 6 hours.The pink coloured supernatent was withdrawn and its optical density was recorded with a spectrophotometer at 485 nm wave length using a blue filter.The dehydrogenase activity was then recorded in terms of triphenyl formazan formed and expressed as µg $TPFh^{-24}$ g^{-1} soil (Klein and Goulding, 1971)

Plant growth characterer

The average number of pods per plant, pod weight and number of nodules was recorder at the time of maturity of the crop.

Nitrogen uptake and indicies

The nitrogen uptake in grain and plant biomass was estimated after digesting the grain and plant sample by concentrated sulphuric acid (H_2SO_4) in presence of digestion mixture.The following formullae were used for calculating the nitrogen uptake

$$\text{Nitrogen uptake(g) in grain per plant} = \frac{\text{percent nitrogen concentration in grain plant}^{-1}}{100} \times \text{dry weight of grains per plant (g)}$$

$$\text{Nitrogen uptake(g) in plant} = \frac{\text{percent nitrogen concentration plant}^{-1}}{100} \times \text{dry weight of plant (g)}$$

The apparent nitrogen recovery and percent soil nitrogen utilization was determined with the help of following formullae

$$\text{Apparent nitrogen recovery (ANR)} = \frac{U_t - U_0}{N_a} \times 100$$

$$\text{Percent soil nitrogen utilization (PSNU)} = \frac{U_t}{N_a + A_N} \times 100$$

Where, U_t = uptake of nitrogen in test treatment (kg/ha), U_0 = uptake of nitrogen in control (kg/ha), N_a = Nitrogen applied to test treatment (kg/ha), A_N = available soil nitrogen (kg/ha).

Statistical analysis

The data was statistically analysed by the method as described by Panse and Sukhatame (1985).

RESULTS AND DISCUSSION

The physico,biochemical and microbiological analysis of the rhizosphere soil samples revealed that the pH and EC (dSm^{-1}) of all the samples was normal, while as significantly maximum organic carbon (1.04%), phosphorus (16.72 kg ha^{-1}), dehydrogenase activity (68.7 µg TPF/24 hr g^{-1} soil), total viable bacteria (78.90 ×10^6), fungi (48.73×10^3), actinomycetes (27.20×10^3), PSB (18.30×10^5) and VAM spores (4.10 g^{-1} soil) was reported from the rhizosphere soil samples collected from the organic farm. The rhizosphere soils collected from kupwara were at par with that of organic farm soils with respect to available phosphorus and total VAM spore population. Similarly although non significant but maximum available nitrogen (298.7 kg ha^{-1}) and potassium (296.3 kg ha^{-1}) was also recorded from the organic farm soils (Tables 1 and 2). The most probable reason for the improved, organic carbon content, available nutrients and biological parameters in the organic farm soils may be the continuous and exclusive use of manures, biofertilizers, biopesticides and botanicals for nutrient pest and management. The management

Table 1: Physico– chemical properties of rhizosphere soils of beans

Sampling location	PH	EC (dSm^{-1})	OC (%)	Available N (kg/ha)	Available P (kg/ha)	Available K (kg/ha)	Dehydrogenase activity (µg TPF/24 hr g^{-1} soil)
Organic farm	7.02	0.24	1.04	298.7	16.72	296.30	68.7
Pulwama	7.30	0.31	0.92	291.3	15.24	248.7	57.2
Baramulla	7.40	0.45	0.84	279.6	13.60	247.2	45.8
Kupwara	7.14	0.29	0.97	293.0	15.81	252.3	62.7
Bandipora	7.30	0.30	0.92	289.5	14.18	251.9	54.2
Anantnag	7.32	0.31	0.90	281.7	14.02	248.4	53.1
SE±	0.22	-	0.003	28.00	0.32	22.00	0.54
CD (0.05)	0.64	ns	0.009	84.02	0.95	65.21	1.63

Table 2: Microbiological properties of rhizosphere soils of beans

Sampling location	Population (cfu/g dry weight of soil)				VAM spores per gram soil
	Total viable bacteria ($\times 10^6$)	Total viable fungi ($\times 10^3$)	Total viable actinomycetes ($\times 10^3$)	Total viable PSB ($\times 10^5$)	
Organic farm	78.90	48.73	27.20	18.30	4.10
Pulwama	68.10	38.81	23.10	12.3	2.6
Baramulla	59.74	40.30	18.12	10.8	2.0
Kupwara	72.60	45.76	24.3	14.8	3.8
Bandipora	65.20	39.48	21.6	11.7	2.4
Anantnag	62.00	41.61	20.4	11.3	2.1
SE±	0.64	1.03	0.34	0.30	0.11
CD (0.05)	1.88	1.09	1.01	0.94	0.36

history of other studied soils revealed the heavy use of synthetic agrochemicals which might have adversly affected the soil health. These findings are in confirmity with the results of Gupta et al. (1980) who reported that organic carbon content of soils showed highly significant and positive correlation with the bacterial and fungal population. The dehydrogenase activity which acts as an indicator of microbiological redox system and is considered as a good measure of overall microbial activity and an index of soil microbial biomass. The maximum content of dehydrogenase in organic farm soils may be due to the highest organic carbon content which supports the microbial population. These findings are supported by the results of Stanislaw and Barbara (2012) who reported that presence of higher level of organic material significaltly increased the soil dehydrogenase activity.

Production of plant growth promoting substances by the Rhizobium isolates

The Rhizobium isolates isolated from the bean plants grown at different locations were screened for the production of various growth promoting sunstances like IAA, GA and siderophore. Screening of the Rhizobium isolates for the production of these plant growth promoting substances showed that all the Rhizobium isolates produced plant growth promoting substances (Table 3), but significantly maximum IAA (39.50), GA(162) and siderophore catechol type 2-3 DHBA was produced by the isolate *Rhizobium phaseoli* (OF). Similar results have been reported by Humphry (2007) who reported that Rhizobia species

Table 3: Production of plant growt promoting substances by *Rhizobium* isolates

Isolates	IAA	GA	Siderophore catechol type 2-3 DHBA
Rhizobium phaseoli (OF)	39.20	162	24
Rhizobium phaseoli (P)	24.18	112	16
Rhizobium phaseoli (B)	37.50	153	21
Rhizobium phaseoli (K)	18.74	96	14
Rhizobium phaseoli (BP)	26.11	148	17
Rhizobium phaseoli (AG)	14.28	84	12
SE±	0.32	1.24	0.35
CD (0.05)	1.02	3.78	0.99

have the ability to produce variable quantity of different plant growth promoting substances like IAA, gibberlic acid,cytokinins etc.The production of auxins, abscisic acid, cytokinins, gibberellins and siderophore is believed to be one of the most important mechanism influencing the growth of plant (Zahir et al., 2004).

Impact of inoculation of *Rhizobium* isolates on plant growth and yield attributes of french bean

The Rhizobium strains after isolation were screened for their plant growth promoting activities and the most effecient one was used in a field experiment in combination with the different levels of inorganic nitrogen to study their integrated effect on plant growth and yield attributes in french beans.

The field experiment results (Table 4) revealed that the number of pods per plant was significantly increased

Table 4: Impact of inoculation of *Rhizobium* isolates on plant growth and yield attributes of french bean

Isolates	Average no. of pods plant^{-1}	Average pod weight (g)	Average no. of nodules plant^{-1}	Nitrogen uptake in grains (kg/ha)	Nitrogen uptake in plant biomass (kg/ha)	Total N uptake (kg/ha)	Apparent nitrogen necovery	Percent soil nitrogen utilization
T_1 :Control	7.94	3.41	35.18	41	64	105		
T_2: N_{20}	9.0	4.0	42.27	55	73	128	115	40.37
T_3: N_{40}	9.0	4.24	38.33	58	77	135	75	40.06
T_4: *Rhizobium phaseoli* (OF)	11.0	4.82	59.12	56	75	131	-	
T_5: *Rhizobium phaseoli* (OF)+N_{20}	12.0	5.96	60.45	63	84	147	210	46.37
T_6: *Rhizobium phaseoli* (OF)+N_{40}	12.0	5.0	58.0	64	84	148	107.5	43.91
SE±	0.39	0.21	0.48	0.72	1.07	1.9	-	-
CD (P=0.05)	0.98	0.53	1.06	1.82	2.74	4.71	-	-

Initial available Nitrogen=297 kg/ha

by every treatment in comparision to control (7.94 pods plant^{-1}). The sole application of 20 kg Nha^{-1} (T_2) and 40 kg Nha^{-1} were at par by producing 9 number of pods plant^{-1}. The maximum number of pods (12 plant^{-1}) were recorded from the treatment combinations T_5: *Rhizobium phaseoli* (OF)+N_{20} and T_6: *Rhizobium phaseoli* (OF)+N_{40}. These findings are in confirmity with the results obtained by Otieno et al. (2009) who reported that starter/low dose of fertilizer nitrogen would not adversly affect nodulation, but would benefit the legume when cotlydon nitrogen is depleted and fixed nitrogen is still unavailable, while as the higher level of fertilizer nitrogen significantly reduced the nodulation.

A good number of nodules (35.18 plant^{-1}) were also found in the control plots which reflected the presence of indigenous isolates capable of nodule formation (Table 4). The nodulation process was significantly increased by every treatment with the maximum number of nodules (60.45 plant^{-1}) recorded from the plants which received treatment T_5:(*Rhizobium phaseoli* (OF)+N_{20}). The nodulation revealed that inoculation of *Rhizobium* isolate with lower level of nitrogen has been more effective as compared to the higher level of inorganic nitrogen. Similar findings were observed by Tahir et al. (2009) who reported nodule number increased significantly by 25 kg N ha^{-1} when applied in combination with *Rhizobium* inoculation in soybean. This significant increase in number of nodules due to inoculation reflects the better combining and symbiotic relationship between introduced rhizobia and French bean. However, the nodule formation was significantly reduced by the application of higher level of nitrogen (N_{40}) either alone or in combination with *Rhizobium*, indicating that higher levels of nitrogen decreased the efficiency of *Rhizobium* inoculation (Tahir et al., 2009).

There was a significant increase in the nitrogen uptake in grain under all the treatments over control (41 kg ha^{-1}) (Table 4). Application of 40 kg N ha^{-1} (T3) and *Rhizobium* inoculation (T4) alone were at par with respect to the nitrogen uptake in grain. Similarly the maximun value of nitrogen uptake in grain (64 kg ha^{-1}) was recorded from treatment T_6: *Rhizobium phaseoli* (OF)+N_{40} but it was at par with treatment T_5: *Rhizobium phaseoli* (OF)+N_{20}. The similar pattern of nitrogen uptake in case of plant biomass was followed with a maximium of 84 kg ha^{-1} observed under treatments T_5: *Rhizobium phaseoli* (OF)+N_{20} and T_6: *Rhizobium phaseoli* (OF)+N_{40}. Consequently the total nitrogen uptake was also significantly improved by all the treatments with maximum value (148 kg ha^{-1}) recorded from the treatment T_6:*Rhizobium phaseoli* (K)+N_{40} (Table 4).. Increase in N uptake in grain and total plant biomass due to *Rhizobium* inoculation alone and in combination with nitrogen fertilizer was mainly because of significant increased in nodulation, which inturn resulted in higher accumulation of N due to atomspheric N_2 fixation. Significant increase in seed and shoot N of soybean inoculated with *Rhizobium strains* was previously reported by Patra et al., (2012) and Zhang et al. (2002).

The tretment T_5: *Rhizobium phaseoli* (OF)+N_{20} recorded maximum apparent nitrogen recovery (210) and percent soil nitrogen utilization (46.37) in comparision to the T_6: *Rhizobium phaseoli* (OF)+ N_{40} (Table 4). This may be due to the reason that higher levels of nitrogen are detrimental to *Rhizobium* strains.These findings are supported by Tahir et al. (2009) and Otieno et al. (2007) who observed that nitrogen fertilizer application significantly reduced nodulation in most of the legume species.

CONCLUSION

The studied soils are biologically quite healthy with presence of efficient Rhizobial strains. The lower is the mineral nitrogen input, the greater is the yield performance of Rhizobium inoculated french bean. The study suggest that higher levels of inorganic nitrogen reduces the performance of efficient Rhizobium strains.

Author Contributions

Z. A. B., designed the study, M. A. A. wrote the article, T. A. S., F. A. S., Z. A. B., S. K., T. S. and B. H. corrected the article.

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