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Effect of Rhizobium Inoculants on Growth Stages and Yield of Pigeonpea in Vertisol

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ABSTRACT: The inoculation of legumes with Rhizobium (Bradyrhizobium) strains selected for high nitrogen fixation under stress conditions of temperature, moisture, and other abiotic and biotic conditions including antibiotics particularly. A research trial was conducted on Effect of *Rhizobium* strains on the growth stages and yield of pigeonpea in Vertisol at the Department of Soil Science, JNKVV, Jabalpur (M.P.) There were twelve treatments (6 exotic, 2 local strain, FUI, UFUI and two Maize control) with four replication laid out in randomized block design. The result shown that *Rhizobium* strain R1 (BRP20) performed best in pigeonpea at different growth stages (25, 45, 65 and 85 DAS) for nodulation attributes (nodule enumeration, its biomass and leg hemoglobin content) by 72.00, 75.00, 60.00 and 34.00%; 63.50, 19.00, 10.30 and 9.70%; and 60.00, 69.00, 72.00 and 69.00% over that of fertilized un inoculated (FUI); plant growth attributes (plant height) by 69.30, 20.90, 22.45 and 24.10%; over that of FUI; and harvest yields (seed and stover) by 16 and 12% over FUI, respectively. The microbial population in rhizospheric soil of pigeonpea increased at 25, 45, 65 and 85 DAS by 1.68, 1.56, 1.75 and 1.54 log fold over that of FUI (Fertilized un inoculated) . For almost every parameters, the treatment of R2 (BRP28), RL1, RL2, R3 (BRP56), R4 (BRP2), R5 (BRP4) and R6 (BRP8) exhibited the performance of next group. The variations on plant parameters for maize crop availing only native microbial benefits were apparently due to genetic attributes of the crop with implication of native microbial influence especially soil existing Rhizobium sp. in the present context and along with that from fertilization.

Keywords: *Rhizobium*, pigeonpea, growth stages, native microbial, Vertisol.

INTRODUCTION

Pigeonpea (*Cajanus cajan* (L.) *Millsp*) is one of the major legume crops of the tropics and subtropics, globally ranking 6th in area and production in comparison to other grain legumes. The crop is relatively drought tolerant, performs well in temperature range of 25-35°C and can also survive at 45°C. The seed of redgram are an important food due to its protein (20-25%), carbohydrates (51-58%) and minerals include calcium, phosphorus, magnesium and vitamin A and C (Odeny, 2007).

One of the major factors which adversely affect pigeonpea productivity is poor nodulation in field due to prevalence of inefficient and poor nodulating native rhizobia (Marsh *et al.*, 2006). found that approximately 77% of the N in the pigeonpea plant was derived from BNF (Sanginga *et al.*, 1996). Although pigeonpea is a

promiscuous legume, results show that *Bradyrhizobium* strains are more efficient for N2 fixation in pigeon pea than *Rhizobium* strains (Anand and Dogra, 1997).

Rhizobium is very good root colonizer it can enhance the growth and yield in leguminous and non-leguminous crops might be due to the diversified activity of *Rhizobium* similar to useful traits of PGPR such as BNF, solubilization of nutrients (N, P, K, Fe, etc), production of phytohormones, BCA (through competitive exclusion, production of anti-microbial compounds and VOC), phyto resistance (ASR and ISR) and tolerance to stress (through anti-oxidants and other bioactive compounds) (Chauhan *et al.*2015 ; Pii *et al.* 2015).

MATERIALS AND METHODS

The present investigation entitled "Efficacy assessment of *Rhizobium* strains on growth, nodulation, yield and rhizobium microbial population in pigeonpea" was carried out during *Kharif* 2019-20.

The experiment was conducted at the Research Field, Department of Soil Science, JNKVV, Jabalpur. Total 12 treatments (including 2 controls as FUI and UFUI for pigeonpea and 2 controls as FUI and UFUI for maize) were tried in four replications under RBD design. The recommended doses of fertilizer @ 25:50:25 kg ha^{-1}(N: P_2O_5: K_2O) and 120:60:40 for the crop (Pigeonpea and maize respectively) were applied in the form of urea, single super phosphate (SSP) and murate of potash (MOP). Seed treatment was done in shade and seeds were sown manually as early as possible.

Table 1: Treatment details.

Treatment	Combination	Crop
T_1	R1 (BRP20)	Pigeonpea
T_2	R2 (BRP24)	Pigeonpea
T_3	R3 (BRP56)	Pigeonpea
T_4	R4(BRP2)	Pigeonpea
T_5	R5 (BRP4)	Pigeonpea
T_6	R6 (BRP6)	Pigeonpea
T_7	RL1	Pigeonpea
T_8	RL2	Pigeonpea
T_9	FUI	Pigeonpea
T_{10}	UFUI	Pigeonpea
T_{11}	FUI	Maize
T_{12}	UFUI	Maize

Growth parameters

Nodulation. Nodulation studies were performed at 25, 45, 65 and 85 DAS of the crops. The rhizospheric soil was washed in gentle running water. Number of nodules were counted and presented in terms of nodules No. $plant^{-1}$. After counting, Nodules were then oven dried in hot air oven at 60°C for (18-20 hrs) 3-4 days till constant weight. The observation on dry weight of nodules in mg $plant^{-1}$ was recorded.

Plant height. Plant height was measured at 25, 45, 65 and 85 DAS. Three plants from each plot were taken and their heights were measured.

Leghemoglobin content of nodules (Wilson and Reisenaur, 1963). Extraction of nodule tissue. The leghemoglobin content was determined with Drabkin's solution and its composition is as under. Take 0.5g of fresh nodule tissue was crushed in a round bottom centrifuge tube with Drabkins solution (3ml). The resultant mixture was centrifuged (15 min at 500xg) so that the large particles of nodule tissue settle down. The supernatant was shifted to a 10 ml volumetric flask. The nodule tissue was extracted twice more and the supernatants were combined with first one in the flask. The total volume was made to 10ml with Drabkins solution, mixed and centrifuged for 30 min at 20000x g. The absorbance was read on Spectronic -20 (Baush and Lomb) at 540nm using Drabkins solution as solvent blank. The amount of leghemoglobin was determined using the calibration factor from standard curve.

Microbial population counts in rhizospheric soil. Samples of rhizospheric soil were used as fresh as possible without grinding, sieving or any modifications. The collected samples in low density polythene bags were stored in refrigerator at 4°C.

Rhizobial population in soil (serial dilution method). For counting population of *Rhizobium* sp. in soil, the study was done adopting serial dilution method. Soil samples were collected periodically for rhizobial study and processed for serial dilution by suspending 10 g of soil sample in 90 ml sterilized water in flasks and were shaken thoroughly which resulted 10^{-1} dilution. Subsequent serial dilutions were made to up 10^{-9} dilution for plating purpose.

Plating (pour method) (David and Davidson, 2014). Plating in sterilized Petriplates were done by taking 1 ml each of 10^{-6} to10^{-9} dilutions as required for *Rhizobial* population counts in soil. Plating was performed in triplicate for each dilution. The composition of YEMA growth medium for *Rhizobium* is as below Table 2.

Table 2: Media composition of YEMA (Yeast Extract Mannitol Agar Medium) for *Rhizobium*.

Ingredient	Quantity $litre^{-1}$
Mannitol	10 g
K_2HPO_4	0.5 g
$MgSO_4.7H_2O$	0.2 g
NaCl	0.1 g
$CaCO_3$	1.0 g
Yeast Extract	1.0 g
Congo red (1:400)	2ml
Agar-agar	15-18 g
Distilled water	1000ml

The serial dilutions obtained from soil samples collected at 25, 45, 65 and 85 DAS of Pigeonpea and Maize were used for plating adopting pour plate method. Soil dilution (10^{-1} to 10^{-7}) aliquot of 1ml was taken in a Petri plate, to this 15 ml of sterilized melted

YEMA medium was poured within the aseptic environment of laminar air flow chamber. After pouring of medium, plates were rotated gently clockwise and anticlockwise to mix evenly the soil dilution with the medium. After solidification of medium the plates were incubated upside down at 28±2°C for 3-7 days. The colonies of *Rhizobium* with specific growth characteristics (round shape with convex elevation mucilaginous) were counted.

$$\text{Viable cells (cfug}^{-1}\text{ soil)} = \frac{\text{Number of colonies}}{\text{Oven dry weight of soil(1g)}} \text{ X Dil. factor}$$

Seed and stover yields. Crop was harvested and bundles were made plot wise, then allowed to dry in the respective plots for 2-3 days and weighed. During threshing, plot wise yields for straw and seeds were recorded separately.

RESULTS AND DISCUSSION

Plant Attributes

Nodules enumeration. The nodules enumeration in pigeonpea given in Table 3. At 25 DAS varied from 13 to 29 No. plant^{-1} with an average of 21 No. plant^{-1}.The highest number of nodules 29 No. plant^{-1} with 72% response over that of FUI (17 No. plant^{-1}) was recorded due to inoculation of isolate R1 (BRP20), followed by R2 (BRP28), RL1, RL2 respectively. At 45 DAS nodules enumeration varied from 18 to 38 No. plant^{-1} with an average of 28 No. plant^{-1}. The isolate R1 (BRP20) exhibited maximum number of nodules 38 No. plant^{-1} with 75% response over that of FUI (22 No. plant^{-1}), followed by that of R2 (BRP28), RL1, RL2, R3 (BRP56) and R4 (BRP2) respectively. The number of nodules. At 65 DAS of the crop nodule ranged from 30 to 46 No. plant^{-1} with the mean value of 38 nodules plant^{-1}. The highest number of nodules of 46 No. plant^{-1} was counted with the treatment of R1 (BRP20) which was 60% more over that of FUI (32 No. plant^{-1}). This was followed by the influence of R2 (BRP28), RL1, RL2 and R3 (BRP56) respectively. At 85 DAS, the effectiveness of *rhizobial* isolates on nodules enumeration ranged from 37 to 60 No. plant^{-1}with an average 51 No. plant^{-1}. The isolate R1 (BRP20) exhibited maximum nodules enumeration of 60 No. plant^{-1} with 34% response over that of FUI (45 No. plant^{-1}), followed by that of R2(BRP28), RL1, RL2, R3 (BRP56) and R4 (BRP2) respectively. Fuhrman and Wollum (1989) reported that co- inoculation of siderophore producing *Pseudomonas* with mixtures of the competing *Bradyrhizobia* typically enhanced nodulation by *Bradyrhizobium japonicum* strain USDA 110. Although the inoculants of exotic rhizobial strains into soil already containing its native population frequently results in production of only a small proportion of nodules containing the introduced strain. This is due to competition with ineffective indigenous strains and other rhizospheric bacteria.

Table 3: Effect of *Rhizobial* isolates on nodules enumeration of pigeonpea at different stages of growth.

Treatments	(Nodules plant^{-1})			
	25 DAS	45 DAS	65 DAS	85 DAS
R1 (BRP20)	29	38	46	60
R2 (BRP28)	26	33	43	57
R3 (BRP56)	21	29	40	53
R4 (BRP2)	20	28	38	52
R5 (BRP4)	19	27	35	51
R6 (BRP8)	17	26	34	49
RL1	24	31	42	55
RL2	23	30	41	54
FUI	17	22	32	45
UFUI	13	18	30	37
Maize (FUI)	0	0	0	0
Maize (UFUI)	0	0	0	0
Mean (Arhar)	21	28	38	51
$SE_m\pm$	1.781	1.891	2.692	1.691
$CD_{5\%}$	5.12	5.51	6.01	4.81

Biomass of nodules of Pigeonpea at different growth stages. The data pertaining tondule number was presented in Table 4 revealed that the nodule number at 25 DAS, the biomass of nodules was increased from 18.00 to 30.30 mg plant^{-1} with the mean value of 23.80 mg plant^{-1}.The isolate R1 (BRP20) influenced nodules biomass for 30.30 mg plant with 63.5% increase over that of FUI (18.50 mg plant^{-1}), followed by that of isolates R2(BRP28), RL1, RL2, R3 (BRP56) and R4 (BRP2) respectively . At 45 DAS, nodules biomass of

pigeonpea varied from 72.50 to 86.30 mg plant with an average of 78.10 mg plant^{-1} .The maximum response in increasing nodules biomass of 86.3 mg plant was recorded due to R1 (BRP20) for 72.50 mg plant^{-1} biomass and 19% response over that of FUI, followed by the effects of R2 (BRP28), RL1, RL2 R3 (BRP56) and R4 (BRP2) respectively. At 65 DAS, the nodules dry biomass varied from 142.50 to 160.30 mg plant^{-1} with an average of 151.70 mg plant^{-1}. Among all the isolates, the isolate R1 (BRP20) recorded for maximum nodules biomass of 160.30 mg plant^{-1} with 10.3% response over that of FUI (145.30 mg plant-^{1}), followed by the influence from R2 (BRP28), RL1, RL2, R3 (BRP56) and R4 (BRP2) respectively At 85 DAS, the nodule biomass ranged from 159.30 to 178.80 mg plant^{-1} with average of 170.20 mg plant^{-1}.The highest performance in nodule biomass of 178.80 mg plant^{-1} was recorded with R1 (BRP20) with 9.7% response as compare to that of FUI (163 mg plant-1). This was followed by the effects from R2 (BRP28), RL1, RL2, R3 (BRP56) and R4 (BRP2) respectively. This result was also reported that rhizobial inoculation improved pigeonpea nodule biomass (Chemining *et al.*, 2007). Significant increase in nodule number, nodule fresh and dry weight was due to increased N and P availability. Availability of more number of *Bradyrhizobium* in the rhizosphere inoculated through seed treatment and also by the continuous supply of P in soil stimulated the multiplication of *Rhizobia* and development of their motile forms which were essentially required for migration through the soil towards the root system. They probably helped in the formation of a greater number of nodules and maintain the higher activity of bacteria in the nodules. These results were in conformation with earlier findings in blackgram (Prabhakaran *et al.*, 2000; Balyan *et al.*, 2002), cowpea (Verma *et al.*, 2013), and green gram (Perveen *et al.*, 2002).

Table 4: Effect of *Rhizobial* isolates on nodules biomass of pigeonpea at different growth stages.

Treatments	Nodules biomass (mg plant^{-1})			
	25 DAS	45 DAS	65 DAS	85 DAS
R1 (BRP20)	30.30	86.30	160.30	178.80
R2 (BRP28)	27.80	82.30	157.50	176.00
R3 (BRP56)	24.50	79.30	154.30	172.50
R4 (BRP2)	23.30	77.80	152.00	171.80
R5 (BRP4)	21.80	76.50	147.50	166.00
R6 (BRP8)	21.30	75.50	147.30	167.50
RL1	26.80	82.00	155.50	174.00
RL2	25.50	81.00	154.80	173.00
FUI	18.50	72.50	145.30	163.00
UFUI	18.00	68.00	142.50	159.30
Maize (FUI)	0.00	0.00	0.00	0.00
Maize (UFUI)	0.00	0.00	0.00	0.00
Mean (Arhar)	23.80	78.10	151.07	170.20
$SE_m\pm$	1.169	1.491	1.406	2.386
$CD_{5\%}$	3.17	4.26	4.02	6.82

Content of leghemoglobin in nodules of Pigeonpea at different growth stages. The content of leghemoglobin given in Table 5. At 45 DAS, leghemoglobin content ranged from 0.80 to 3.25 mg g^{-1} nodules with mean value of 2.5 mg g^{-1} nodules. The exotic isolate of R1 (BRP20) performed maximum for leghemoglobin content of 3.25 mg g^{-1} nodules and 69% response over that of FUI (1.92 mg g^{-1} nodules), followed by the response from R2 (BRP28), RL1, RL2 and R3 (BRP56) for the leghemoglobin content of 3.17, 3.09, 2.92 and 2.82 mg g^{-1} nodules along with of 65.00, 61.00, 52.00 and 47.00% response, respectively over that of FUI. At 65 DAS**,** the content of leghemoglobin in nodules of the leguminous crop varied from 1.03 to 4.63 mg g^{-1} nodules with an average of 3.40 mg g^{-1} nodules.R1 (BRP20) responded maximum for leghemoglobin content of 4.63 mg g^{-1} nodules which was 72% higher over that of FUI (2.68 mg g^{-1} nodules), followed by the effects of R2 (BRP28), RL1 and RL2 with leghemoglobin content of 4.25, 4.23 and 4.08 mg g^{-1} nodules and 59.00, 58.00 and 52.00% response, respectively.

The findings of Deka and Azad (2006) was also in conformation that the leghemoglobin has a positive correlation with N-fixation and nitrogenase activity in nodules characteristics of efficient symbiosis. Kaur *et al.*, (2015) reported that dual co-inoculation of *Bradyrhizobium* sp. with PGPR2, PGPR3, and LK884 showed significant improvement in leghemoglobin content over *Mesorhizobium* sp. alone in chickpea. Because, heme prosthetic group for plant leghemoglobin was provided by the bacterial symbiont within symbiotic group.

Table 5. Effect of *Rhizobial* isolates on content of leghemoglobin in nodules of Pigeonpea at different stages of growth.

Treatment	Leghemoglobin content (mg g^{-1} nodules)	
	45 DAS	65 DAS
R1 (BRP20)	3.25	4.63
R2 (BRP28)	3.17	4.25
R3 (BRP56)	2.82	3.65
R4 (BRP2)	2.65	3.35
R5 (BRP4)	2.27	3.05
R6 (BRP8)	1.96	2.98
RL1	3.09	4.23
RL2	2.92	4.08
FUI	1.92	2.68
UFUI	0.80	1.03
Maize (FUI)	0.00	0.00
Maize (UFUI)	0.00	0.00
Mean (Arhar)	2.50	3.40
$SE_m\pm$	0.293	0.402
$CD_{5\%}$	0.83	1.14

Height of crops at different growth stages. In Table 6 the plant height of pigeonpea at 25 DAS was increased from 14.50 cm to 31.75 cm with the mean value of 24.73 cm. The isolate R1 (BRP20) recorded for maximum plant height of 31.75 cm with 69.3% increment relative to that of FUI (18.75 cm), followed by that of R2 (BRP28), RL1, RL2, R3 (BRP56) and R4 (BRP2), the exhaustive crop of maize (FUI) attained a plant height of 33.25 and 28.50 cm due to FUI and UFUI, respectively which are apparently more relative to that of controls (FUI and UFUI) of pigeonpea, as of from genetic attribute, while the conditions were same for both the crops. At 45 DAS height of pigeonpea varied from 50.50 to 66.50 cm with an average value of 59.03 cm .The exotic isolate of R1 (BRP20) responded maximum for the pigeonpea plant height of 66.5 cm with 20.9% response as compared to that of FUI (55.00 cm), followed by the ensuring performing group of R2 (BRP28), RL1, RL2, R3 (BRP56), and R4(BRP2) While**,** maize **(**FUI) gained the plant height of 64.50 and UFUI for 62.50 cm. At 65 DAS, The effect of different strains on plant height of pigeonpea varied from 92.75 to 120.00 cm with the mean value of 110.26 cm. The significantly maximum plant height of 120 cm was recorded with the isolate R1 (BRP20) which was 22.45% more as compared to that of FUI (100.50 cm). This was followed by the effects of R2 (BRP28), RL1, RL2, R3 (BRP56) and R4 (BRP2) While, maize gained plant height of 127.50 and 124.25 cm with FUI and UFUI, respectively. At 85 DAS, the plant height of pigeonpea ranged from 130 to 166 cm with an average value of 152.44 cm. The isolate of R1 (BRP20) influenced the best for maximum plant height of166.00 cm with 24.1% response over that of FUI (133.75 cm), followed by the response from R2 (BRP28), RL1, RL2, R3 (BRP56) and R4 (BRP2) While the plant height of maize was 188.50 and 174.50 cm with FUI and UFUI, respectively. HALDI and Bano (2010), observed that the effect of diazotrophs which fix atmospheric N_2 into ammonium ions on lead (Pb) phytoextraction and their subsequent effect on the growth of maize (*Zea mays* L.) *Rhizobium leguminosarum* strain TAL-102 and *Azotobacter chrococum* ware used as single culture as well as co-culture resultant increase in plant growth and biomass.

Table 6: Effect of *Rhizobial* isolates on plant height of crops at different stages of growth.

Treatment	Plant height (cm)			
	25 DAS	45 DAS	65 DAS	85 DAS
R1 (BRP20)	31.75	66.50	120.00	166.00
R2 (BRP28)	29.75	62.50	110.75	152.00
R3 (BRP56)	26.75	59.75	107.25	147.75
R4 (BRP2)	25.50	59.00	106.25	146.25
R5 (BRP4)	23.00	58.00	105.25	144.50
R6 (BRP8)	22.00	57.00	103.25	144.00
RL1	28.25	61.25	109.50	149.50
RL2	27.00	60.75	109.00	148.25
FUI	18.75	55.00	98.00	133.75
UFUI	14.50	50.50	92.75	130.00
Maize (FUI)	33.25	64.50	127.50	188.50
Maize (UFUI)	28.50	62.50	124.25	174.50
Mean (Arhar)	24.73	59.03	110.26	152.44
$SE_m\pm$	1.495	1.251	2.290	4.349
$CD_{5\%}$	4.30	3.60	6.50	12.40

Yields (seed and stover) of crops (pigeonpea and maize) at harvest. The data on seed and stover yield of pigen pea (Table 7) revealed that the seed yield of pigen pea differed significantly among all the treatments The isolate of R1(BRP20) significantly influenced seed yield for 1850 kg ha^{-1} which was 16% more as compared to that of FUI (1593 kg ha^{-1}), followed by the ensuing performing group of R2 (BRP28), RL1, RL2, R3 (BRP56) and R4 (BRP2). While maize crop yielded 1765 and 1733 kg ha^{-1} seed due to FUI and UFUI, respectively. Similar was the pattern of results in case of stover yield of pigeonpea. It ranged from 6355 to 7455 kg ha^{-1} with a mean value of 6836 kg ha^{-1}. The isolate of R1 (BRP20) responded for maximum yield of stover for 7455 kg ha^{-1} along with 12% increment as compared to that of FUI (6653 kg ha^{-1}), followed by the performance of R2 (BRP28), RL1, RL2, R3 (BRP56) and R4 (BRP2). Stover yielded of maize was recorded 4693 kg ha^{-1} with fertilization of FUI and 4375 kg ha^{-1} with unfertilization of UFUI. Seed inoculation of *Rhizobium*+PSB could record record the highest HI (24.93%) followed by individual inoculation of *Rhizobium* (24.28%) in pigeon pea (Machetele and Kushwaha (2011**)**. This might happen due to increased economic yield with induced changes in seed proteome and metabolome involved in enhanced resistance level against diseases. The present study revealed that enhanced growth and yield in pigeon pea might be due to the diversified activity of *Rhizobium* similar to useful traits of PGPR such as IAA, flavonoid and siderophore production which enhance root growth and resulting nutrient uptake. Similar to our work various researchers have reported the synergistic effects of phytohormones producing *PGPR and Rhizobium* on nodulation and yield of legumes crop **(**Kaur *et al.*2015). Three isolates of *Rhizobium* sp. (chickpea, berseem and lentil) were screened with and without L-TRP and used in maize field and characterized for different biochemical tests. Three isolates of *Rhizobium* sp. produced IAA equivalents in the absence of L-TRP and increased the value of IAA equivalents in the presence of L-TRP. Results clearly demonstrated improved growth and yield components might be the reason for enhanced growth through *Rhizobium* sp. Zahir *et al.*, (2010).

Table 7: Effect of rhizobial isolates on yields (seed and stover) of crops at harvest.

Treatment	Yield kgha^{-1}	
	Seed	Stover
R1 (BRP20)	1850	7455
R2 (BRP28)	1733	6955
R3 (BRP56)	1713	6856
R4 (BRP2)	1705	6780
R5 (BRP4)	1685	6742
R6 (BRP8)	1683	6731
RL1	1728	6924
RL2	1725	6912
FUI	1593	6653
UFUI	1590	6355
Maize (FUI)	1765	4693
Maize (UFUI)	1733	4375
Mean (Arhar)	1708	6452
$SE_m\pm$	39.018	54.573
$CD_{5\%}$	111.52	155.97

Population of *Rhizobium* sp. in soil of crops (pigeonpea and maize) at different growth stages. The data on *Rhizobium* population in rhizospheric soil of pigeonpea (Table 8) showed that at 25 DAS, the *Rhizobium* population in soils of pigeonpea ranged from 1.63 log cfu (4.26×10^1 log cfu g^{-1} soil) to 5.90 log cfu (79.43×10^4 log cfu g^{-1} soil) with an average of 4.54 log cfu (3.46×10^4 cfu g^{-1} soil).The exotic isolate of R1 (BRP20) influenced for maximum rhizobial population for 5.9 log cfu (79.43×10^4 cfu g^{-1} soil) with 1.68 log fold more over that of FUI (3.5 log cfu = 3.16×10^3 cfu g^{-1} soil) which was due to the native rhizobial population in presence of the leguminous crop. This was followed by the effects of R2 (BRP28), RL1, RL2, R3 (BRP56) and R4 (BRP2) for the diazotrophic population of rhizobial. While, the native rhizobial population in soil of maize (a non-leguminous crop) was 1.75 log cfu (5.62×10^1 cfu g^{-1} soil) and 1.43 log cfu (2.69×10^2 cfu g^{-1} soil) with FUI and UFUI, respectively.

At 45 DAS of pigeonpea, the diazotrophic population varied from 3.00 log cfu (10×10^3 cfu g^{-1} soil) to 6.25 log cfu (17.78×10^5 cfu g^{-1} soil) with the mean value of 4.90 log cfu (7.90×10^4 cfu g^{-1} soil). Among all the isolates, R1 (BRP20) influenced maximum the bacterial population for 6.25 log cfu (17.78×10^5 cfu g^{-1} soil) with 1.56 fold increment over that from FUI (4.00 log cfu = $10x10^3$ cfu g^{-1} soil). The ensuing performing group was R2 (BRP 24), RL1 and RL2 for the rhizobial population. While the native rhizobial population of

2.40 log cfu (2.51 × 10^2 cfu g^{-1}soil) and 2.1 × log cfu (1.25 × 10^2 cfu g^{-1} soil) were found in rhizospheric soil of maize due to FUI and UFUI, respectively.

The data on rhizobial population in soil of pigeonpea at 65 DAS ranged from 4.00 log cfu (10 × 10^3 cfu g^{-1} soil) to 7.00 log cfu (10 × 10^6 cfu g^{-1} soil) and an average of 5.30 log cfu (19.9 × 10^4 cfu g^{-1} soil). The isolate of R1 (BRP20) influenced maximum for population of the diazotroph of 7.00 log cfu (10 × 10^6 cfu g^{-1} soil) with 1.75 log fold response over that of FUI 4.90 log cfu (7.94 × 10^4 cfu g^{-1} soil), followed by the effects of R2 (BRP28), RL1, RL2, R3 (BRP56) and R4 (BRP2) for the rhizobial population. While, maize rhizospheric soil exhibited the bacterial population of 3.29 log cfu (19.4 × 10^2 cfu g^{-1} soil) and 3.2(15.8 × 10^2 cfu g^{-1} soil) due to FUI and UFUI, respectively (Singh *et al.*, 2021). The populations of *Rhizobium* sp. in the rhizosphere of soybean at harvest increased maximum count by 5.98 log cfu (95.28×104 cfu g^{-1} soil) with the relative esponse 2.43 log fold increase over the control FUI (2.46 log cfu = 28.68×101 cfu g^{-1} soil) (Yaduwanshi *et al.*, 2021). Combination of RDF+PGPR + *Actinomycetes* + *Arthrobacter* performed significantly better towards rhizospheric microbial populations at 25, 45 and 65 days after sowing with respect to *Actinomycetes*, *Pseudomonas* and *Arthrobacter*. (Chabot *et al.*, 1996) reported that strain of *Rhizobium leguminosarum* bv. *Phaseoli* and PGPR were examined for the potential of root colonization in maize and lettuce. Rhizobia were superior colonizer compared with other tested bacteria rhizobial root population averaged 4.1 log CFU g^{-1} (fresh wt.) on maize roots 4 weeks after seedling and log 3.7 CFU g^{-1} (fresh wt.) on lettuce roots 5 weeks after seedling. The average populations of the recovered PGPR strains were 3.5 and 3.0 log CFU g-^{1} (fresh wt.) on maize and lettuce roots, respectively. Similar findings were supported by Weaver and Frederick (1972) reporting that increasing the number of rhizobia in soil with its inoculation to soybean plants. The synergistic effect of endophytic bacteria and *Bradyrhizobium* in greengram; N-fixation, P-solubilization, phytohormone production also played a vital role in maintaining microbial population (Hoflich *et al.*, 1995). Another probable reason was due to the availability of N and P which appeared to have encouraged the multiplication of organisms (Saha *et al.*, 1985) The increased root growth indirectly influenced microbial population by increasing the root area of colonization. The increased root metabolic activity also resulted in the increase in the number of nutrients.

Table 8: Effect of different isolates on populations of *Rhizobium* sp. in rhizospheric soil of crops at different stages of growth.

Treatment	*Rhizobial* population (log cfu and in parenthesis cfu g^{-1} soil)		
	25 DAS	45 DAS	65 DAS
R1 (BRP20)	5.90(79.43 × 10^4)	6.25 (17.78 × 10^5)	7.00 (10 × 10^6)
R2 (BRP28)	5.73 (53.7 × 10^4)	5.63 (42.65 × 10^4)	5.80(6.30 × 10^5)
R3 (BRP56)	5.50(31.62 × 10^4)	5.42 (26.3 × 10^4)	5.37 (26.30 × 10^4)
R4 (BRP2)	5.37 (23.4 × 10^4)	5.30 (19.95 × 10^4)	5.30 (19.95 × 10^4)
R5 (BRP4)	3.75 (56.23 × 10^2)	4.65 (4.46 × 10^4)	5.25 (17.7 × 10^4)
R6 (BRP8)	2.75 (5.62 × 10^2)	4.58 (3.8 × 10^4)	5.39 (24.5 × 10^4)
RL1	5.70 (50.11 × 10^4)	5.60(39.81 × 10^4)	5.38 (23.9 × 10^4)
RL2	5.65 (42.65 × 10^4)	5.52 (31.62 × 10^4)	4.90(7.94 × 10^4)
FUI	3.50(3.16 × 10^3)	4.00(10 × 10^4)	4.00(10 × 10^3)
UFUI	1.63 (4.26 × 10^1)	3.00 (10 × 10^3)	3.29 (19.4 × 10^2)
Maize (FUI)	1.75 (5.62 × 10^1)	2.40 (2.51 × 10^2)	3.20(15.8 × 10^2)
Maize (UFUI)	1.43 (2.69 × 10^2)	2.10 (1.25 × 10^2)	2.10 (1.25 × 10^2)
Mean (Arhar)	4.54 (3.46 × 10^4)	4.90(7.9 × 10^4)	5.30 (19.9 × 10^4)
$SE_m\pm$	0.580	0.522	0.120
$CD_{5\%}$	1.65	1.49	0.34

CONCLUSION

It was concluded that maximum number of nodules and its biomass, plant height, seed and stover yield and population of Rhizobium sp. in rhizospheric soil of crops at different stages of growth was recorded with the isolate of R1 (BRP20) over fertilized uninoculated (FUI). The variations on plant parameters for maize crop availing only native microbial benefits were apparently due to genetic attributes of the crop with implication of native microbial influence especially soil existing *Rhizobium* sp. in the present context and along with that from fertilization. While, the variability in native rhizobial population in soil of maize (a non-leguminous crop) was due to the effects of fertilization apparent in direct and reciprocal manner, respectively.

FUTURE SCOPE

- *Rhizobium*, besides an effective symbiotic diazotroph, can also be studied further as rhizobacterium for other leguminous and non-leguminous crops.
- The isolates under the study may be further characterized cytogenetically, serologically and biochemically.
- The study on interaction of the isolates with existing

soil microorganisms (including wild types) may be carried out for the purpose of mass production of biofertilizer to be benefitted to field crops.

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Conflicts of Interest. None.

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