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Mono and Co-inoculation Response of *Rhizobium* and PGPR on Soybean in Central India

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Authors' contributions

This work was carried out in collaboration between all authors. Author FCA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AKR and DLNR managed the analyses of the study. Author FCA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Nitrogen fixation by legume-rhizobium symbiosis is important to agricultural productivity and is therefore of great economic interest. The effect of mono and co-inoculation with *Rhizobium* and PGPR was investigated in two consecutive years during 2010-11 and 2011-12 in Vertisols of central India on soybean. Soybean crop was grown during rainy season of 2010-11 with mono-inoculation of *Rhizobium* (10 isolates) and PGPR (15 isolates). During 2011-12 previously screened 3 taxonomically confirmed isolates each of *Rhizobium* and PGPR as mono-inoculation and their combinations as co-inoculation along with fertilized uninoculated control (FUI) and unfertilized uninoculated control (UFUI) were also evaluated. The treatments were laid out in RCBD with three and four replications respectively. The effects of mono-inoculation and co-inoculation on soybean were observed on soil properties, nodulation, yield, total nutrients (NPK) uptake by crop, harvest index, nitrogen harvest index and additional BNF (biological nitrogen fixation). Seed inoculation either mono or co-inoculation improved the status of post harvest soil nutrients as compared to initial status, UFUI and FUI. During 2010-11 the total soil N in post harvest soil sample was maximum with one of the rhizobial isolates (441 mg kg^{-1} soil) while in FUI it was 402 mg kg^{-1} soil

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while with PGPR isolates it was 439 and 401 mg kg^{-1} soil respectively. Next year it was 432 and 430 mg kg^{-1} soil respectively in co-inoculation and FUI.

Mono-inoculation promoted germination, nodulation, seed yield, harvest index, nitrogen harvest index and additional BNF significantly over FUI but co-inoculation was found more synergistic. Significantly correlations were observed between oven dried weight of nodules with straw and seed yields and total N uptake by crop.

Keywords: Rhizobium; PGPR; co-inoculation; mono-inoculation; FUI; UFUI.

1. INTRODUCTION

Leguminous crops are of great importance throughout the developing world, providing a valuable source of protein in the human diet as well as animal fodder and pollen for honey production. However, they are also key assets for sustainable agriculture, thanks to their root nodules. These nodules contain bacteria called rhizobia, which fix atmospheric nitrogen, making it available to the plant. One consequence of this is a reduction in the plant need for nitrogen fertilizer.Madhya Pradesh is known as soybean state of India and soybean is principal high protein grain legume of Madhya Pradesh is presently grown in about 1.68 million ha area in 2008-09 [1]. Soybean was initially introduced in Madhya Pradesh in mid sixties, and due to absence of native effective rhizobial strains in most of the soils, it was inoculated with bradyrhizobial inoculants imported from USA. Later on, the indigenous biofertilizer production units came into existence in the state, which resulted in increased native Indian strains. Use of such inoculants leads to better establishment of native rhizobial population in the soil [2].

Plant growth in agricultural soils is infused by a myriad of abiotic and biotic factors. Farmers routinely use physical and chemical approaches to manage the soil environment for improving crop yields. The application of microbial inoculants for this purpose is comparatively less common but it influences the plant rhizosphere in a diverse range. Plant root offer a niche for the proliferation of microorganisms that thrive on root exudates and lysates. These rhizospheric microorganisms in turn having a great impact on root biology, nutrients transformation and plant growth promotion. Plant growth promoting rhizobacteria (PGPR) are root associated soil bacteria that facilitate plant growth and development [3,4,5,6,7,8]. The direct promotion of plant growth by PGPR generally entails providing the plant with compounds synthesized by the bacterium or which facilitate uptake of nutrients from the environment. The indirect promotion of plant growth occurs when PGPR's lessen or prevent the deleterious effects of pathogens on plants by production of inhibitory substances or by increasing the natural resistance of the host [9,10,11].

Some bacteria that live in the rhizosphere, referred to as plant growth promoting rhizobacteria (PGPRs), are able to modify nodule formation and biological nitrogen fixation (BNF) when they are co-inoculated with rhizobia [12,13,14]. Co-inoculation leads to an increased number of the most active nodules, therefore, to a greater N_2-fixation and assimilation [15,16].

2. MATERIALS AND METHODS

2.1 Site and Climate

Field experiments were carried out during 2010-11 and 2011-12 at research farm of Department of Soil Science and Agricultural Chemistry, JNKVV, Jabalpur, Madhya Pradesh, India. The site is located at N 23°12'46.6'' and 079°56'47.6'' E with an altitude of 419 meter above mean sea level (MSL) and has a semi – arid subtropical monsoon type climate with hot summers (maximum day temperature variation of 18–33°C and 25–43°C respectively). The total rainfall received during the year was 1621.1 and 1901.8 mm with average relative humidity of 75.1 and 81.8% respectively. The length of the average growing period ranged from 90–100 days. Soybean [*Glycine max* (L.) Merill] cv. JS – 9752 was grown in rainy season (June – October) in both the years.

2.2 Soil Characteristics

Both the experiments were conducted on the same location in a Vertisol with 56.8% clay, 25.3% sand and 17.9% silt. Soil samples of 0 – 15 cm depth were collected from 8 spots in a zig-zag pattern and a initial composite sample was prepared after air drying and grinding for chemical analysis. Plot wise post harvest soil samples were also drawn for the purpose.

Soil pH was determined in a 1:2.5 soil:water (s/w) suspension using KCl glass electrode pH meter and after settling down, the conductivity of supernatant liquid was determined by null method using wheatstone circuit through conductivity meter [17]. Organic carbon was determined by oxidizing the organic matter using chromic acid and the excess of unreduced dichromate was back titrated with standard ferrous ammonium sulphate [18]. Kjeldahl method was used to determine total nitrogen [17]. Available nitrogen ($KMnO_4$ oxidizable N) was oxidized by potassium permanganate and released ammonia was absorbed in boric acid [19]. Available phosphorus in soil was extracted by 0.5 M N_aHCO_3 having 8.5 pH using ascorbic acid as colour development agent [20]. Ammonium acetate (1 N) was used for extracting available potassium and its estimation by flame photometer [21].

Table 1. Physico-chemical analysis of the soil

S. no.	Soil characteristics	Soybean	
		2010-11	2011-12
1.	Ph	7.20	7.11
2.	EC (dSm^{-1})	0.22	0.18
3.	Organic carbon (g kg^{-1})	3.42	3.50
4.	$KMnO_4$oxidizable N (mg kg^{-1})	92	96
5.	Available P (mg kg^{-1})	6	6
6.	Available K (mg kg^{-1})	108	113
7.	Total N (mg kg^{-1})	410	417

2.3 Treatments and Experimental Design

Previously screened (under net house) isolates of *Rhizobium* (10 nos.) and PGPR (15 nos.) were field evaluated along with FUI (fertilized un-inoculated control) and UFUI (unfertilized un-inoculated control) during 2010-11 and the taxonomically identified and shortlisted three isolates of each organisms were further field evaluated during 2011-12 as mono-inocuation and their combinations as co-inoculation under RCBD with three and four replications respectively. All the plots except UFUI received N, P and K through urea, single super phosphate and mureate of potash respectively as par recommendations (20:80:20 kg ha^{-1} for both the years). As par treatments, seeds were inoculated except fertilized and unfertilized uninoculated control. All the treatments were replicated for three and four times respectively using RCBD design.

2.4 Seed Treatment and Inoculation

According to plot size (10 m^2), 75 g of seed for both the years were weighed in clean polythene bags and were moistened with sterilized water. Seeds were first treated with bavistin fungicide (@ 2 g kg^{-1} seed) and were little allowed to air dry. After that 1 ml of gum acacia (2%) sticker solution was poured on the seed of each polythene bag followed by 1 ml of liquid formulations for individual isolate as mono-inoculation and 1 ml of each isolate for their combinations as co-inoculation. Seeds were enough shaken for proper mixing and coating of inoculants. After little air drying in shade, seeds were sown in the field. One set of polythene bag with treated seeds with individual isolate was kept separately and was immediately brought to the laboratory for enumeration of bacterial load $seed^{-1}$ (average counts were $7.5x10^6$ cells $seed^{-1}$ for soybean).

2.5 Nodulation Studies and Seed Yield

Scoring of nodules (no. of nodules $plant^{-1}$ and their oven dried weight) was done at maximum vegetative growth stage (45 days after sowing) by randomly uprooting five plants in each plot taking all precautions to prevent loss of nodules.

After harvest straw and seed yields were recorded and the total nitrogen, phosphorus and potassium uptake was worked out.

3. RESULTS AND DISCUSSION

3.1 Seed Germination

Plant growth promoting rhizobacteria (PGPR) stimulate plant growth by producing phytohormone which enhance the growth and physiological activities of the host plant. Legume bacteria also have the potential for growth stimulation. The results suggested that inoculation (whether through *Rhizobium* or PGPR) collectively increased the seedling emergence significantly and it was 44% and 50% more over FUI by *Rhizobium* and PGPR isolates respectively as mono-inoculation while during 2011-12 it was 14% and 28% more over FUI due to mono and co-inoculation group respectively (Tables 2, 4 and 6). *Pseudomonas* showed the good compatibility with *R. japonicum* [22,23].

3.2 Nodulation

Nodules number was also enhanced significantly due to inoculation and it was more by *Rhizobium* group (59%) than PGPR group (22%) over FUI during 2010-11. While during 2011-12 it was

45% and 34% more due to mono and co-inoculation group respectively over FUI but the oven dried nodular mass was more by co-inoculation group (86%) as compared to mono-inoculation group (71%) over FUI which clearly indicate that the size of effective nodules has got more importance even if they are less in number (Tables 2, 4 and 6). There is mutualistic symbiotic association between legume and rhizobia, which results in the formation of nitrogen fixing sites on the roots of legumes. In addition to other factors, plant harmones have an important role in developing and establishing nodules. Some PGPR promote nodulation by lowering endogenous levels of plant harmone ethylene in roots through their aminocyclopropane-carboxylate (ACC) -deaminase activity. So co-inoculation might have increased the nodular oven dried [24,14, 25,26,27,28,29,30].

3.3 Yields and Total Nutrient Uptake by Crop

Individual inoculation by ten rhizobial and fifteen PGPR isolates collectively gave soybean seed yield of 2523 kg and 2595 kg ha^{-1} which was 14% and 18% more over FUI respectively while the straw yield was 5112 kg and 5204 kg ha^{-1} and it was 19% and 12% higher over FUI (Tables 2 and 4). During 2011-12 average performance of rhizobial and PGPR isolates as mono-inoculation was 2571 kg and 5316 kg ha^{-1} towards seed and straw yields which was 20% and 8% higher to FUI while that of co-inoculation it was 2749 and 5752 kg ha^{-1} which was 29% and 16% higher over FUI. It clearly indicate that the co-inoculation practice increased the seed yield by 9% over mono-inoculation (Table 6). Increase in seed and straw yields due to co-inoculation was probably due to increase in proportion of effective nodules, supply of N and P and synthesis of growth hormones. The observed promotion in seed yield in this study could be attributed to cumulative effects of these rhizobacteria. Similar results were obtained by [31,32,33,34,35].

Seed and straw yields were also found positively correlated (R^2=0.716 and R^2=0.585 respectively) with oven dried nodular mass (Fig. 1a and b).

Total nutrients (NPK) uptake by crop was significant either with mono or co-inocualtion practices over fertilized uninoculated control. Taking into account the average response of mono and co-inoculation practices towards total nutrient (NPK) uptake by crop it was 276, 16.01, 180 kg ha^{-1} and 344, 18.7, 196 kg ha^{-1} respectively by mono and co-inoculation practice respectively and it was 25%, 17% and 9% more by co-inoculation over mono-inoculation respectively (Table 7). Total nitrogen uptake by crop reflected positive correlation (R^2=0.629) with oven dried weight of nodules (Fig. 1c). Co-inoculation of phosphate solubilizing bacteria (PSB) *Pseudomonas* sp. and *B. japonicum* significantly increased nodulation, plant total N, P uptake, seed yield and yield components of soybean over no inoculation and chemical fertilizers alone [36]. [37] Reported that the dual inoculation of *Bradyrhizobium japonicum* and phosphate solubilizing bacteria significantly increased nodulation, seed and biomass yield, nutrient uptake and symbiotic N fixation. Combined inoculation with N_2-fixing and phosphate-solubilizing bacteria was found to be more effective than inoculation with single microorganism by providing a more balanced nutrition for plants [38]. Dual inoculation increased the yield in black gram [39].

3.4 Harvest Index and Nitrogen Harvest Index and Additional BNF

On the basis of data received through experimentation, harvest index, nitrogen harvest index and additional BNF was worked out for both the experimental years considering grain and straw yields, nitrogen uptake by grain and the total nitrogen uptake by the crop (grain + straw) over FUI respectively (Tables 2, 4 and 7). Harvest index is the ratio between total biomass and economic yield. Pulses generally exhibit low harvest index as compared with cereals. While considering the average percentage of harvest and nitrogen harvest indexes of both the crops and groups (mono and co-inoculation) it was found that they are almost identical but these parameters were higher to FUI and UFUI while additional BNF was comparatively more with co-inoculation groups (68%) as compared to mono-inoculation group. [40] Reported that parameters like biological yield and harvest index are closely related to sink size, source activity and sink source ratio. [41] Reported that photosynthesis, dark reaction and the partitioning of assimilates are the essential prerequisite for increased and stable plant productivity. [42] Found that seed inoculation with *Rhizobium* significantly increases plant height, LAI, number of pods per plant, number of seeds per pod, 1000 seed weight, TDM, seed yield and harvest index in soybean.

Table 2. Effect of *B. japonicum* isolates on germination, nodulation, grain and straw yield and total NPK uptake by soybean crop, harvest and nitrogen harvest index and additional BNF

Treatments	Germination % 4th DAS	Nodulation		Yield (kg ha^{-1})		Total uptake (kg ha^{-1})			Harvest index (%)	Nitrogen harvest index (%)	Additional BNF (kg ha^{-1})
		Nodules no. plant^{-1}	ODW (g plant^{-1})	Grain	Straw	N	P	K			
R_{11}	50±2.1d	21.9±0.8c	0.18±0.009b	2845±77c	5780±101c	3 16±6.74d	22±0.55d	226±4.82e	32.97±0.27a	63±0.39a	17 3±6.84c
R_{12}	25±2.2b	20.3±0.9c	0.16±0.011b	2533±144b	5103±279b	276±13.75c	17±0.70c	196±11.50c	33.17±0.71a	63±1.01a	125±17.39b
R_{14}	23±2.4a	17.8±2.6b	0.15±0.018b	2503±191b	5065±417b	248±20.53c	16±1.23b	183±15.80c	33.12±0.49a	64±1.31a	89±25.80a
R_{16}	29±2.2b	19.2±2.5b	0.13±0.032b	2623±120b	5258±285b	283±12.44d	17±0.85c	202±10.11d	33.31±0.27a	64±0.84a	131±17.25b
R_{27}	25±2.1b	18.8±1.0b	0.13±0.019b	2403±90b	4735±166a	2 21±6.76b	14±0.34b	172±7.79b	33.66±0.19a	64±0.46a	63 ±7.90a
R_{30}	43±3.1c	16.3±2.3b	0.09±0.019a	2543±93b	5185±104b	2 57±9.20c	15±0.25b	184±5.41c	32.88±0.57a	68±2.08b	10 0±13.46a
R_{33}	24±0.6a	15.7±1.3b	0.09±0.006a	2510±97b	5085±187b	2 61±15.21c	16±0.57b	184±6.90c	33.04±0.12a	64±1.63a	1 06±21.09a
R_{34}	23±0.8a	18.1±0.8b	0.12±0.015a	2470±166b	4895±260b	242±16.98c	15±0.93b	171±8.70b	33.48±0.48a	67±0.71a	86±22.01a
R_{35}	48±3.2c	19.7±1.1b	0.14±0.007b	2628±115b	5270±183b	286±6.89d	17±0.54c	200±7.48d	33.27±0.90a	64±1.18a	1 37±6.45a
R_{51}	28±2.6b	17.8±1.0b	0.15±0.017b	2473±141b	4740±288a	247±15.81c	16±1.34b	174±10c	34.30±0.46a	63±1.21a	89 ±18.92a
FUI	22±1.5a	11.7±1.3a	0.08±0.017a	2220±45a	4290±169 a	198±5.67b	12±0.18a	148±4.50b	34.18±1.33a	69±1.93b	0
UFUI	19±1.0a	10.7±0.6a	0.07±0.003a	1998±106a	4035±3 82a	138±6.14a	10±0.78a	120±10.46a	33.36±1.18a	75±2. 02c	-51
SEd	2.34	2.01	0.02	142.14	345.86	15.4	0.98	11.36	0.94	1.81	19.74
CD (p=0.05)	5.10	4.37	0.05	309.72	753.62	33.6	2.13	24.75	2.04	3.94	43.02
CV (%)	11.14	16.40	26.23	8.11	9.87	8.81	8.80	8.93	3.96	3.88	32.01

Table 3. Effect of *B. japonicum* isolates on soil chemical properties after harvest of soybean crop

Treatments	pH	EC (dSm^{-1})	OC (g kg^{-1})	Total N (mg kg^{-1})	Available nutrients (mg kg^{-1})			Chlorophyll index (SPAD value)
					N	P	K	
R_{11}	7.8±0.06b	0.22±0.003a	3.80±0.04c	441±0.6f	125±2.1c	10.9±0.1f	157±1.0d	41±0.12e
R_{12}	7.7±0.02a	0.21±0.016a	4.13±0.06c	428±1.1e	121±0.9c	10.6±0.1f	155±0.9d	40±0.12d
R_{14}	7.8±0.03b	0.24±0.005b	3.53±0.41b	421±2.2d	93±3.5b	9.1±0.2d	143±2.6c	39±0.13c
R_{16}	7.7±0.07a	0.21±0.008a	3.80±0.10b	421±3.7d	119±1.3c	9.4±0.3e	143±3.8c	39±0.13c
R_{27}	7.7±0.01a	0.22±0.005a	3.87±0.28a	420±1.8d	89±3.9a	8.8±0.2d	143±1.6c	39±0.05c
R_{30}	7.7±0.08a	0.21±0.005a	3.43±0.41a	417±5.6d	95±2.3b	9.1±0.4d	138±1.5c	39±0.14c
R_{33}	7.8±0.04b	0.21±0.004a	4.37±0.53b	422±2.7d	99±2.7b	9.8±0.1e	148±2.3d	40±0.09d

Treatments	pH	EC (dSm^{-1})	OC ($g\ kg^{-1}$)	Total N ($mg\ kg^{-1}$)	Available nutrients ($mg\ kg^{-1}$)			Chlorophyll index (SPAD value)
					N	P	K	
R_{34}	7.8±0.03b	0.21±0.003a	3.57±0.72b	386±3.4b	91±2.7a	8.7±0.2c	143±2.6c	39±0.18c
R_{35}	7.6±0.09a	0.22±0.010a	3.70±0.02c	433±3.1e	118±2.4c	10.5±0.1f	152±1.6d	40±0.05d
R_{51}	7.7±0.04a	0.21±0.003a	3.83±0.98b	419±3.1d	100±6.8b	10.4±0.1f	152±1.9d	40±0.14d
FUI	7.7±0.06a	0.21±0.005a	4.44±0.26a	402±1.5c	85±1. 6a	8.0±0.1b	118±7.0b	35±0.18b
UFUI	7.8±0.03b	0.22±0.005a	4.03±0.30a	352±2.0a	81±3 .4a	6.7±0.3a	87±5.5a	33±0.23a
SEd	0.05	0.01	0.63	4.15	4.44	0.22	4.02	0.20
CD (p=0.05)	0.12	0.02	1.27	9.04	9.68	0.47	8.77	0.43
CV (%)	1.01	6.67	19.74	1.42	6.20	3.29	4.07	0.73

Table 4. Effect of PGPR isolates on germination, nodulation, grain and straw yield and total NPK uptake by soybean crop, harvest and nitrogen harvest index and additional BNF

Treatments	4^{th} DAS	Nodulation		Yield ($kg\ ha^{-1}$)		Total uptake ($kg\ ha^{-1}$)			Harvest index (%)	Nitrogen harvest index (%)	Additional BNF ($kg\ ha^{-1}$)
		Nodules no. $plant^{-1}$	ODW ($g\ plant^{-1}$)	Grain	Straw	N	P	K			
P_2	73±1.3d	27±1.4b	0.17±0.01b	2765±7b	5735±27c	289±14 d	17±0.18c	202±2.65d	33±0.76	50±1.61	74±1.61c
P_3	83±1.0f	25±2.5b	0.17±0.01b	2300±9a	4533±10a	238±10 b	16±0.28b	176±1.80b	34±1.04	58±2.65	24±1.76a
P_4	72±1.0d	25±1.5b	0.15±0.01a	2740±18b	5427±18b	288±1 0d	17±0.38c	191±3.28c	34±0.76	58±0.76	73±2.18c
P_{10}	73±1.8d	22±1.7a	0.16±0.02b	2740±51b	5505±37c	264±1 5d	17±0.41c	191±2.18c	33±1.50	53±2.52	50±2.25b
P_{17}	75±1.3e	26±1.0b	0.17±0.02b	2517±45a	5167±34b	262±1 2c	15±0.29b	175±1.53b	33±1.04	56±1.73	47±2.52b
P_{25}	70±0.8d	27±2.8b	0.19±0.03c	2600±35a	5230±35b	289±2 0d	17±0.28c	181±2.52c	33±1.61	54±1.61	75±2.08c
P_{26}	80±1.3f	24±2.3b	0.18±0.02b	2499±22a	4828±36a	264±1 1c	15±0.26b	184±0.76c	34±0.50	54±1.76	49±1.04b
P_{33}	77±1.8e	24±1.4b	0.18±0.01b	2590±41a	5237±1b	254±10 b	17±0.74c	188±3.75c	33±1.32	55±1.76	40±2.02a
P_{48}	82±1.3f	25±2.5b	0.19±0.01c	2780±41b	5553±44c	268±2 4c	18±0.76c	192±1.73c	33±1.00	55±2.18	54±1.26b
P_{55}	78±1.3e	25±1.2b	0.17±0.02b	2217±53a	4433±41a	247±1 1b	15±0.50b	172±1.44b	33±0.76	55±2.02	32±2.00a
P_{68}	82±1.5f	22±2.3a	0.20±0.01c	2712±32b	5288±36b	267±9c	17±0.59c	191±3.06c	34±1.04	57±2.08	52±1.80b
P_{70}	73±1.3d	22±2.0a	0.17±0.01b	2712±55b	5388±10b	287±1 5d	18±0.57c	190±0.76c	34±1.26	53±2.50	73±1.26c
P_{72}	63±1.3c	24±2.0b	0.17±0.02b	2813±33b	5520±44b	268±1 0c	18±0.50c	192±3.61c	34±1.89	55±2.25	54±2.65b
P_{179}	70±1.3d	26±3.0b	0.15±0.01a	2397±34a	4937±46b	240±2 4b	15±0.29b	190±1.26c	33±1.53	53±2.18	26±2.65a
P_{249}	79±1.5e	22±2.6a	0.20±0.03c	2550±40a	5283±15b	247±1 4b	18±0.76c	190±2.25c	33±1.15	53±2.08	32±2.00a
FUI	58±1.3b	20±1.7a	0.14±0.01a	2192±4a	4642±41a	214 ±3a	13±0.38a	153±3.28a	32±1.00	53±1.89	0.00
UFUI	52±1.0a	20±1.0a	0.13±0.01a	2180±29a	4500±44a	2 08±4a	12±0.76a	146±3.61a	30±1.26	48±1.76	-12±1.00
SEd	2.15	1.55	0.01	240	223	9.5	0.7	3.9	3.0	3.3	9.4
CD (p=0.05)	4.40	3.16	0.02	490	455	19.4	1.5	8.0	NS	NS	19.2
CV (%)	3.62	7.82	7.21	12	5	4.5	5.5	2.6	10.9	7.4	24.4

Table 5. Effect of PGPR isolates on soil chemical properties after harvest of soybean crop

Treatments	pH	EC (dSm^{-1})	OC ($g\ kg^{-1}$)	Total N ($mg\ kg^{-1}$)	Available nutrients ($mg\ kg^{-1}$)			Chlorophyll index (SPAD value)
					N	P	K	
P_2	7.73±0.05	0.17±0.01	3.99±0.28i	437±11.88e	98±2.75a	7.11±0.07b	131±7.49d	39±0.02e
P_3	7.53±0.18	0.22±0.02	3.89±0.13g	423±3.68c	108±7.86a	6.75±0.04a	127±0.58c	37±0.01c
P_4	7.73±0.12	0.22±0.01	3.62±0.18d	416±4.87b	110±2.84a	7.51±0.09b	136±9.12e	39±0.01e
P_{10}	7.75±0.09	0.19±0.02	4.09±0.11c	414±4.09b	98±4.69a	6.84±0.04a	126±8.98c	37±0.01c
P_{17}	7.73±0.09	0.21±0.02	3.82±0.10f	412±5.04b	95±1.77a	7.15±0.06b	131±15.39d	36±0.02b
P_{25}	7.77±0.09	0.18±0.01	3.95±0.05d	427±5.45c	117±3.70a	8.80±0.24d	159±12.82h	39±0.01e
P_{26}	7.70±0.09	0.16±0.01	3.92±0.08h	439±7.87e	121±2.19b	8.89±0.21d	169±11.75i	37±0.02c
P_{33}	7.73±0.05	0.21±0.01	3.62±0.13j	429±8.89d	106±6.39a	8.80±0.10d	152±10.0j	37±0.01c
P_{48}	7.73±0.07	0.14±0.01	4.02±0.03k	400±3.34a	94±2.80a	6.44±0.28a	121±5.77	40±0.02f
P_{55}	7.75±0.07	0.23±0.01	3.95±0.53b	425±4.47c	111±7.10a	7.69±0.09b	137±6.43e	39±0.02e
P_{68}	7.72±0.07	0.19±0.02	3.85±0.26e	437±10.22e	108±3.02 a	7.87±0.16c	140±10.77f	38±0.01d
P_{70}	7.72±0.09	0.23±0.01	4.05±0.36f	413±16.23b	97±6.27a	6.93±0.22b	127±5.97c	38±0.01d
P_{72}	7.58±0.06	0.20±0.01	3.62±0.15j	424±4.05c	98±8.67a	7.07±0.07b	127±9.58c	41±0.02g
P_{179}	7.65±0.09	0.16±0.01	3.75±0.17j	437±6.81e	106±5.37a	8.04±0.15c	140±17.35f	36±0.01b
P_{249}	7.63±0.09	0.19±0.01	3.85±0.18e	415±4.65b	108±5.57a	7.20±0.14b	132±6.93d	37±0.01c
FUI	7.75±0.09	0.17±0.01	3.59±0.23b	401±2.29a	104±5. 25a	6.75±0.23a	126±11.51c	34±0.04a
UFUI	7.70±0.08	0.16±0.01	3.52±0.10a	399±8.50a	90±2. 00a	6.22±0.03a	116±7.47a	36±0.07b
SEd	0.06	0.002	0.65	2.9	12.8	0.3	1.4	0.03
CD (p=0.05)	NS	NS	1.31	5.9	26.2	0.7	2.9	0.06
CV (%)	1.33	1.13	20.77	1.4	6.7	2.5	2.6	0.09

Table 6. Effect of mono and co-inoculation with previously shortlisted Rhizobium and PGPR isolates on germination, nodulation, grain and straw yields by soybean crop

Treatments	Germination (%) at 4th DAS	Nodulation		Yield ($kg\ ha^{-1}$)	
		Nodules no. $plant^{-1}$	ODW ($g\ plant^{-1}$)	Grain	Straw
R_{27}	68±5.2a	31±3.0b	0.22±0.02a	2482±106b	5147±212b
R_{33}	66±3.8a	29±6.3b	0.24±0.02b	2552±102b	5404±351b
R_{35}	70±5.0a	31±3.3b	0.29±0.03b	2483±132b	5286±568b
P_3	64±5.9a	25±4.5a	0.26±0.04b	2660±100b	5561±409b
P_{10}	72±5.7a	28±2.7b	0.28±0.03b	2750±122b	5538±288b
P_{25}	70±6.0a	30±3.1b	0.25±0.02b	2500±95b	4962±178b
$R_{27}+P_3$	65±5.3a	27±3.1b	0.22±0.02a	2720±125b	5405±253b
$R_{27}+P_{10}$	78±3.8b	23±1.4a	0.24±0.03b	2761±105b	6216±244c
$R_{27}+P_{25}$	74±6.8a	33±1.4b	0.31±0.02b	2690±120b	5431±287b

Treatments	Germination (%) at 4th DAS	Nodulation		Yield (kg ha^{-1})	
		Nodules no. plant^{-1}	ODW (g plant^{-1})	Grain	Straw
$R_{33}+P_3$	86±3.8b	28±2.6b	0.27±0.01b	2660±124b	5367±306b
$R_{33}+P_{10}$	82±6.8b	24±1.5a	0.30±0.01b	2778±47b	5852±309c
$R_{33}+P_{25}$	76±8.3b	24±3.9a	0.29±0.04b	2764±69b	5649±126b
$R_{35}+P_3$	70±7.1a	31±3.2b	0.28±0.06b	2697±121b	6017±191c
$R_{35}+P_{10}$	85±3.6b	24±1.4a	0.29±0.02b	2860±50c	6175±239c
$R_{35}+P_{25}$	75±9.1b	27±2.2b	0.31±0.04b	2810±66c	5656±56b
FUI	60±4.0a	20±1.2a	0.15±0.04a	2139±91a	4941±417b
UFUI	58±6.0a	18±1.7a	0.14±0.03a	1897±99a	3794±199a
SED	8.40	4.31	0.04	148.03	419.37
LSD (p=0.05)	16.97	8.72	0.08	299.17	847.54
C.V. (%)	16.57	22.94	23.05	8.05	10.91

Table 7. Effect of mono and co-inoculation with previously shortlisted *Rhizobium* and PGPR isolates on total NPK uptake by soybean crop, harvest and nitrogen harvest index and additional BNF

Treatments	Total uptake (kg ha^{-1})			Harvest index (%)	Nitrogen harvest index (%)	Additional BNF (kg ha^{-1})
	N	P	K			
R_{27}	272±14c	15.5±0.2b	174±12.0c	32.53±0.27b	43.55±0.5a	96±14.0a
R_{33}	280±15c	15.8±0.7b	181±11.9c	32.20±1.21b	45.84±2.0a	104±15.3a
R_{35}	273±24c	15.8±0.7b	169±11.7c	32.25±1.23b	47.48±1.2a	97±24.0a
P_3	276±16c	16.3±1.0b	191±10.6c	32.50±0.91b	46.55±2.3a	101±15.8a
P_{10}	297±19c	16.9±1.1b	192±13.1c	33.20±0.32c	46.78±1.8a	121±19.4a
P_{25}	258±19c	15.8±1.3b	172±10.4c	33.50±0.06c	47.33±1.8a	82±19.5a
$R_{27}+P_3$	335±10d	18.2±0.6c	187±10.5c	33.47±0.05c	47.74±3.4a	159±9.7b
$R_{27}+P_{10}$	364±23d	18.8±0.9c	206±5.4d	30.78±0.90a	46.05±1.4a	1 89±23.0c
$R_{27}+P_{25}$	316±21c	18.1±1.1c	184±11.8c	33.15±0.30c	47.50±2.1a	140±20.6b
$R_{33}+P_3$	304±17c	17.2±0.9b	182±6.4c	33.18±0.31c	47.61±1.1a	1 28±17.4a
$R_{33}+P_{10}$	379±17e	19.7±1.1c	196±8.1c	32.28±0.86b	49.81±2.8b	2 04±16.5d
$R_{33}+P_{25}$	336±16d	19.2±0.7c	195±9.7c	32.85±0.35b	46.76±1.3a	1 61±15.6b
$R_{35}+P_3$	338±15d	18.0±0.8b	209±10.8d	30.93±0.87b	46.37±3.3a	162±14.7b
$R_{35}+P_{10}$	386±14e	20.7±0.8d	213±5.1d	31.70±0.56b	47.92±1.2a	2 11±17.2c
$R_{35}+P_{25}$	340±12d	18.4±0.7c	193±2.9c	33.18±0.31c	50.53±2.3b	1 65±12.0b
FUI	176±11b	12.1±0.6a	135±11.6b	30.41±1.06a	44.33±1.2a	00
UFUI	120±9a	9.8±0.7a	103±5.1a	28.78±0.75a	44.10±0.6a	-55
SED	24.00	1.25	14.06	1.00	2.58	24.05
LSD (p=0.05)	48.60	2.52	28.41	2.03	5.21	48.60
C.V. (%)	11.45	10.48	10.96	4.38	7.79	28.01

Table 8. Effect of mono and co-inoculation with previously shortlisted *Rhizobium* and PGPR isolates on soil chemical properties after harvest of soybean crop

Treatments	pH	EC (dSm^{-1})	OC ($g\ kg^{-1}$)	Total N ($mg\ kg^{-1}$)	Available nutrients ($mg\ kg^{-1}$)		
					N	P	K
R_{27}	7.14±0.02a	0.22±0.01a	3.4±0.01b	409±4.97a	104±1.49a	9.11±0.33b	136±3.63b
R_{33}	7.20±0.02b	0.21±0.01a	4.5±0.03e	419±3.34a	102±1.69a	9.33±0.68b	140±4.42b
R_{35}	7.19±0.01b	0.27±0.02c	3.1±0.03a	428±6.36b	103±2.44a	9.67±1.06b	137±3.82b
P_3	7.12±0.01a	0.20±0.02a	4.3±0.02e	426±7.11b	110±2.84b	8.33±0.61b	125±3.85a
P_{10}	7.24±0.01c	0.22±0.02a	4.1±0.03d	420±6.36a	101±2.75a	7.55±0.57a	135±4.61b
P_{25}	7.10±0.01a	0.23±0.01b	3.6±0.06b	424±3.50b	105±3.12a	8.33±0.47b	139±5.42b
$R_{27}+P_3$	7.13±0.01a	0.19±0.01a	4.6±0.11f	429±4.97b	110±2.56b	7.89±0.27a	139±2.65b
$R_{27}+P_{10}$	7.14±0.01a	0.20±0.01a	3.4±0.02b	424±5.54b	109±1.75b	9.11±1.13b	137±4.60b
$R_{27}+P_{25}$	7.13±0.01a	0.19±0.01a	3.7±0.12c	427±7.04b	108±2.78b	8.11±0.76b	133±2.76b
$R_{33}+P_3$	7.14±0.01a	0.20±0.01a	3.3±0.03a	431±3.71b	106±0.94b	7.33±0.43a	135±4.05b
$R_{33}+P_{10}$	7.21±0.01b	0.20±0.01a	4.0±0.06d	426±4.68b	106±1.77b	8.33±0.49b	140±5.66b
$R_{33}+P_{25}$	7.17±0.01b	0.23±0.01b	4.0±0.01d	417±3.96a	104±2.55a	8.22±0.59b	141±2.42b
$R_{35}+P_3$	7.20±0.02b	0.21±0.01a	3.9±0.08c	430±2.99b	109±2.32b	7.55±0.48a	138±3.76b
$R_{35}+P_{10}$	7.22±0.01c	0.21±0.01a	4.0±0.20d	432±5.13b	105±3.37a	7.67±0.38a	138±4.90b
$R_{35}+P_{25}$	7.13±0.01a	0.20±0.01a	4.3±0.01e	419±2.71a	101±5.59a	8.33±0.36b	134±4.99b
FUI	7.13±0.01a	0.20±0.01a	4.1±0.03d	430±2.72b	98±4.74a	6.78±0.35a	125±3.79a
UFUI	7.12±0.01a	0.19±0.01a	3.6±0.06b	421±2.90b	97±2.15a	6.22±0.41a	117±4.85a
SED	0.02	0.02	0.10	6.87	4.08	0.87	5.92
LSD (p=0.05)	0.04	0.03	0.20	13.86	8.25	1.75	11.97
C.V. (%)	0.35	10.69	3.60	2.29	5.51	15.10	6.22

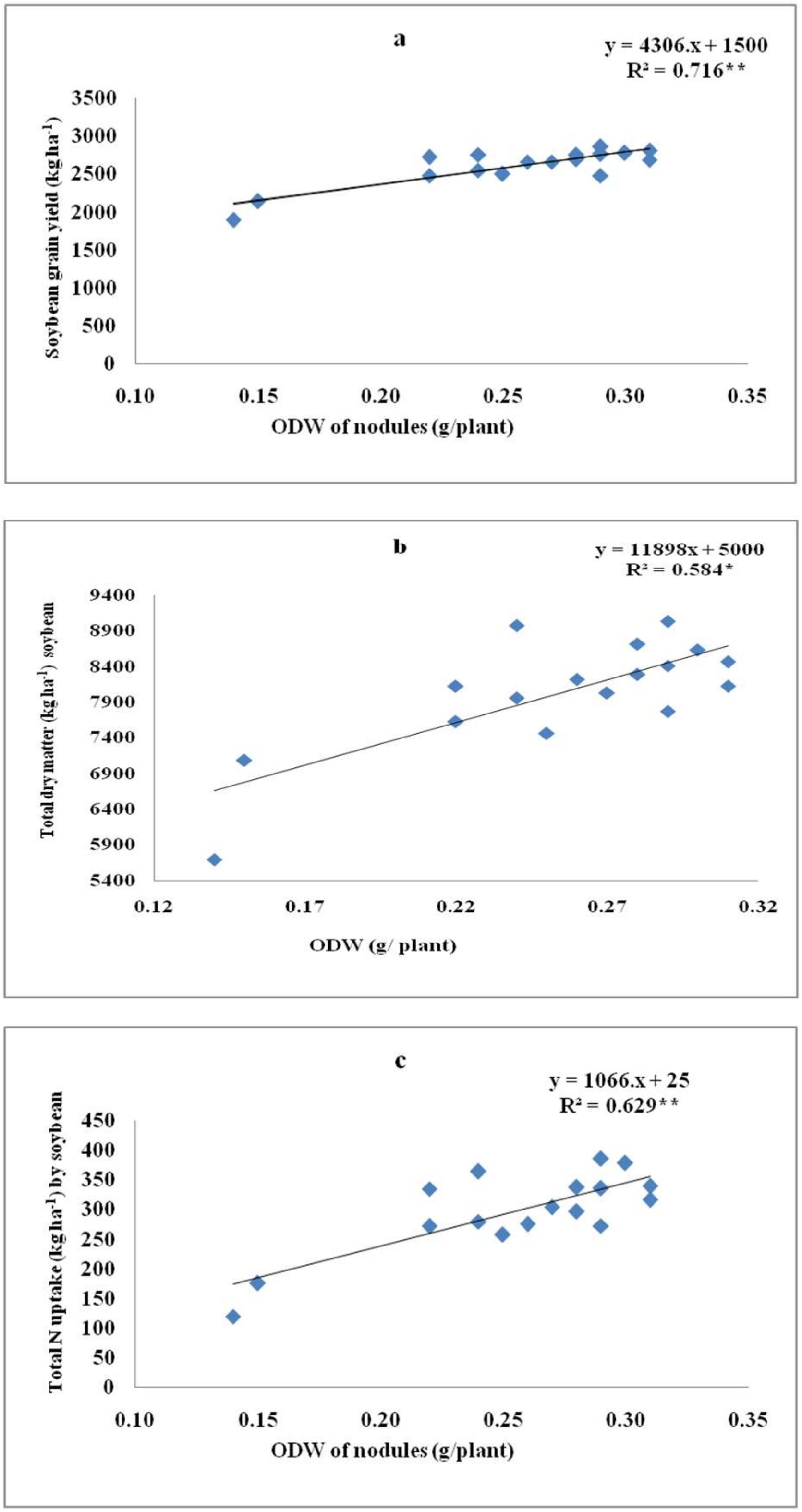

Fig. 1. Correlation between ODW of nodules: grain yield, straw yield and total nitrogen uptake by crop

3.5 Post Harvest Soil Properties

Among different treatments no significant differences could be finding with pH, EC and organic carbon but there was good appreciation in organic carbon as compared to initial one. Average of individual group of isolates (*Rhizobium* and PGPR) significantly increased the SOC over FUI. Inoculation practice made positive impact towards increasing the available nutrients (NPK) and total N status of soil when compared with initial status (Tables 3 and 5). Co-inoculation of *Rhizobium* and PGPR isolates was better as compared to their mono-inoculation towards SOC and available NPK in soil during 2011-12 but the increase was non-significant (Table 8). There was increase of 5 mg total N kg^{-1} soil due to co-inoculation over mono-inoculation. Microorganisms involved in P solubilization enhance plant growth by increasing the efficiency of biological nitrogen fixation and enhancing the availability of plant nutrients by the production of plant growth promoting substances [43,44].

4. CONCLUSION

Seed inoculation either mono or co-inoculation improved the status of post harvest soil nutrients as compared to initial status. The total soil nitrogen in post harvest soil sample was maximum with one of the rhizobial isolates (441 mg N kg^{-1} soil) over FUI (402 mg N kg^{-1} soil). In the next season the nitrogen content was found 432 and 430 mg kg^{-1} soil respectively with co-inoculation and FUI. The Mono-inoculation promoted germination, nodulation, seed yield, harvest index, nitrogen harvest index and additional BNF significantly over FUI but co-inoculation was found more synergistic. The seed yield of soybean increased by 9% with co-inoculation practice over mono-inoculation. Significant correlations were observed between oven dried weight of nodules with straw, seed yields and total N uptake by crop. Co-inoculation of *Rhizobium* and PGPR isolates was better as compared to their mono-inoculation towards SOC and available NPK in soil.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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