International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(6): 2094-2102

Received: 19-09-2017
Accepted: 20-10-2017

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Co-inoculation response of *Rhizobium* and PGPR on soybean and chickpea

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Abstract
Field experiments were carried out during 2011-12 in Vertisols of central India on soybean-chickpea cropping sequence. Soybean and chickpea crops were grown in *kharif* and *rabi* seasons respectively with 17 treatments (previously screened 3 best 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) using RBD with four replications. Co-inoculation of rhizobia with plant growth promoting rhizobacteria (PGPR) played an important role in both promotion of nodulation and plant growth of leguminous crops. In this study, the effect of mono-inoculation and co-inoculation on soybean and chickpea were observed on soil properties, nodulation, yield, total nutrient uptake by crop, harvest index, nitrogen harvest index and additional BNF (biological nitrogen fixation). Status of major available soil nutrients was found better in the post harvest soil samples after both the crops (soybean and chickpea) which received co-inoculation (*Rhizobium* + PGPR) as compared to mono-inoculation. 14 kg soil available N was found as left over N by soybean crop for chickpea. Co-inoculation played a synergistic role with native rhizobacteria for promotion of nodulation, better additional BNF (biological nitrogen fixation), seed yield, harvest index and nitrogen harvest index of soybean and chickpea due to co-inoculation of seed as compared to mono-inoculation, FUI and UFUI. Significant correlations for both the crops between total nitrogen uptake, soybean grain and straw yield with oven dried weight of nodules were also observed.

Keywords: *Rhizobium*, PGPR, Co-inoculation, Mono-inoculation, FUI and UFUI

Introduction
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 influence 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. (Ditchfield, 1993 [15]; Mantelin and Touraine, 2004 [31]; Poonguzhali *et al*., 2006 [40]; Hiroyuki *et al*., 1998 [28]; Glick, 1995 [20]; Glick *et al*., 1999 [21]). 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 (Handelsman and Stabb, 1996 [26]; Cartieaux *et al*., 2003 [12]; Kloeeper *et al*., 1988 [29].)

Material and methods
Site and Climate
Field experiments were carried out during 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^{o}12’46.6’’ and 079 ^{o}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 25 – 43^{o}C) and mild winters (7 - 25^{o}C). The total rainfall received during the year was 1901.8 mm with average relative humidity of 81.8 %. The length of the growing period ranged from 90 – 100 and 150 – 160 days for soybean and

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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 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 setting down, the conductivity of supernatant liquid was determined by null method using wheatstone circuit through conductivity meter (Piper, 1967) [39]. 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 (Walkey and Black, 1934) [52]. Kjeldahl method was used to determine total nitrogen (Piper, 1967) [39]. Available nitrogen ($KMnO_4$ oxidizable N) was oxidized by potassium permanganate and released ammonia was absorbed in boric acid (Subbiah and Asija, 1956) [49]. Available phosphorus in soil was extracted by 0.5 M N_aHCO_3 having 8.5 pH using ascorbic acid as colour development agent (Adams, 1974) [2]. Ammonium acetate (1 N) was used for extracting available potassium and its estimation by flame photometer (Muhr *et al*., 1963) [34].

Soil characteristics of the experimental site before sowing of soybean and chickpea.

S. No.	Soil characteristics	Soybean	Chickpea
1.	pH	7.11	7.13
2.	EC (dSm^{-1})	0.18	0.20
3.	Organic carbon (g kg^{-1})	3.50	3.70
4.	$KMnO_4$oxidizable N (kg ha^{-1})	216	230
5.	Available P (kg ha^{-1})	13.5	14.5
6.	Available K (kg ha^{-1})	254	240
7.	Total N (kg ha^{-1})	938	950

Treatments and experimental design

Both the experiments with soybean and chickpea were carried out with 17 treatments (previously screened 3 best isolates each of *Rhizobium* (soybean and chickpea) and PGPR as mono-inoculation and their combinations as co-inoculation along with fertilized uninoculated control (FUI) and unfertilized uninoculated control (UFUI) using RBD with four replications. All the plots except UFUI received N, P, K and Zn through urea, single super phosphate, mureate of potash and zinc sulphate respectively as par recommendations (20:80:20 and 5 kg ha^{-1} for both the crops i.e. soybean and chickpea). As par treatments, seeds were inoculated except fertilized uninoculated control. All the treatments were replicated thrice using RBD design.

Seed treatment and inoculation

According to plot size (10 m^2), 75 g of seed for both the crops 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 and $6.9x10^6$ cells $seed^{-1}$ for chickpea)

Nodulation studies and seed yield

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

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

Results

Seed germination

Soybean

At 4^{th} day of sowing the germination was highest (72 %) with P_{10} among *Rhizobium* and PGPR isolates followed by P_{25} and P_{35} (70 %) against FUI (60 %). Co-inoculation with R_{33} + P_3 gave the highest germination percentage (86 %) but rest of the combinations were statistically at par to it except R_{27} + P_3 (Table 1). Co-inoculation resulted in better germination as compared to mono-inoculation. *Pseudomonas fluorescens* showed the best compatibility with *B. japonicum* among tested beneficial microorganisms (Belkar and Gade, 2012) [8].

Chickpea

Mono-inoculation of PGPR isolate P_{10} gave highest germination percentage (68%) among all the treatments of mono and co-inoculation and it was the only treatment which was found significant over FUI. Average performance of mono and co-inoculation was also found identical (58 %) with chickpea crop (Table 3). Rudresh *et al*., (2005) [44] also noticed that the chickpea seed germination, nutrients uptake, plant height, nodulation, pea yield and total biomass was increased due to combined inoculation practice.

Nodulation

Soybean

More number of nodules was found with *Rhizobium* as compared to PGPR isolates but the difference was not significant. Co-inoculation did not give much impact on number of nodules plant^{-1}. Oven dried weight of nodules plant^{-1} was increased significantly due to all treatments except R_{27} and R_{27}+P_3 over FUI but on considering the average performance of individual inoculation (0.26 g plant^{-1}) against co-inoculation (0.28 g plant^{-1}) not much difference could be observed (Table 1). This is in agreement with previous reports demonstrating the beneficial effects of PGPR belonging to *Pseudomonas* spp. and *Azospirillum* spp. on symbiotic efficiency of rhizobia nodulating different legume crops (Dashti *et al*., 1998 [13]; Parmar and Dadarwal, 1999 [37]; Singer *et al*., 2000 [46]; Figueiredo *et al*., 2007 [18]).

Co-inoculation studies with PGPR and *Rhizobium* / *Bradyrhizobium* spp. have been shown to increase root and shoot biomass, nodule dry matter, nitrogenase activity, N_2-fixation and grain yield in legumes (Elkoca *et al*., 2008 [17], Thi Thi et. al., 2013 [51]). Under field conditions, *Azospirillum* sp. co-inoculation with either *B. japonicum* CB 1809 or USDA 110 gave 32.23 and 16.85% nodulation, and 26.51 and 18.83% nodule dry weight increase over single inoculation of CB 1809 and USDA 110, respectively. Nodulation showed positive correlation with yield components such as number of pods per plant as a result of *Bradyrhizobium japonicum* inoculation, which in turn reduces the use of P fertilizer in soybean production (Son *et al.*, 2006) [48]. A mutualistic symbiosis, of both ecological and agronomical importance occurs between leguminous plants and rhizobia, involving the development of a specialized plant organ, the root nodule (Brevin, 1991) [10].

Chickpea

Number of nodules with chickpea crop did not reflect any significant changes over FUI except P_{10} but PGPR isolate P_{25} was found significant over UFUI. The average scoring of mono and co-inoculation group was identical (17 and 16 nodules plant^{-1} respectively). With regard to oven dried weight of nodules all of the treatments were significantly superior over FUI except R_{40} and P_{25} but on considering the average response of individual and co-inoculants it was 0.12 and 0.21 mg plant^{-1} respectively (Table 3). Co-inoculation of *Rhizobium* spp. and *Azospirillum* spp. can increase the number of root hairs, the amount of flavonoids exuded by the roots and the number of nodules formed as compared to single *Rhizobium* inoculation (Remans *et al*., 2008) [42].

Rhizospheric microorganisms may not only influence the inoculated rhizobia adversely through saprophytic competition, but also help them in survival through synergism resulting in an increase in their nodulation ability and N_2-fixing efficiency (Gupta *et al*., 2003) [23]. The supply of P to host plant stimulated the multiplication of rhizobia and essentially their movement through the soil towards the root system thereby improving the nodulation (White, 1953 [53]; Madhok, 1961 [30]). In legume root nodules, IAA which is produced by most of PGPR, activates the enzyme H+ATP-ases, which is fundamental for energy production in the nodules (Rosendahl and Jochimsen, 1995) [43]. It is also well known that plant root flavonoids are the inducers of nodulation gene (nod genes) expression in *Rhizobium* (Maxwell *et al*., 1989 [33]; Peter and Verma, 1990 [38]).

Yields and total nutrient uptake by crop

Soybean

Grain yield was significantly increased by all the treatments over fertilized uninoculated control whether the crop was inoculated by individual isolates or their combinations as co-inoculation. Considering the average impact of mono-inoculation against co-inoculation it was 2571 kg ha^{-1} and 2749 kg ha^{-1} respectively which is found to be 7 % more due to co-inoculation practice over mono-inoculation, while it was 8% higher with straw yield (Table 1). Grain and total dry matter yields were found positively correlated (R^2=0.716 and 0.584 respectively) with oven dried weight of nodules (Figs. 1. a and b). Inoculation with mixed culture of *B. japonicum* containing either *Azotobacter vinelandii* or *Azospirillum brasilense* gave increased yields in soybean (Herschkovitz *et al*., 2005) [27]. Plants inoculated with Rb-133+*P. fluorescens* P-93 gave the highest seed yield, which showed 50% increase as compared to *Rhizobium* alone (Yadegari and Rahmani, 2010) [54].

Co-inoculation significantly increased the seed yield by 23.65 and 34.92% over single inoculation of CB 1809 and USDA 110, respectively, and three to six times over non-inoculated control (Thi Thi *et al*., 2013) [51]. *Bradyrhizobium japonicum* forms a symbiotic relationship with soybean (*Glycine max*) and gives an increase in nodulation which leads to increases in plant fresh weight, seed protein and seed yield (Smith and Hume, 1987) [47].

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 25%, 17% and 9% respectively more by co-inoculation over mono-inoculation (Table 1). Total nitrogen uptake by crop reflected positive correlation (R^2=0.629) with oven dried weight of nodules (Fig. 1. c). 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 negative control and chemical fertilizers (Argaw, 2012) [5]. Abdalla and Omar (2001) [1] 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 (Belimov *et al*., 1995) [7]. Dual inoculation increased the yield in black gram (Tanwar *et al*., 2002) [50].

Chickpea

Chickpea grain and straw yields were statistically significant by all the treatments whether *Rhizobium* and PGPR isolates were used individually or in different combinations. The average performance of these two groups towards grain yield was 2181 kg ha^{-1} and 2298 kg ha^{-1} respectively and the co-inoculation gave 5 % higher grain yield over mono-inoculation while with straw yield it was 6 % (Table 3). Bacterization of green gram with the composite inoculants of *Rhizobium* sp., *Pseudomonas fluorescens* and *Bacillus megaterium* were highly beneficial in enhancing the plant growth and yield of green gram besides effecting a reduction in the cost of inorganic fertilizers. (Anandaraj and Leema, 2010). Plant growth-promoting rhizobacteria (PGPR) are beneficial native soil bacteria that colonize plant roots and result in increased plant growth (Glick, 1995 [20]; Cleyet-Marcel *et al*., 2001 [11]; Branex *et al*., 2005 [9]). Grain and total dry matter yields were positively correlated (R^2=0.523 and 0.640 respectively) with oven dried weight of nodules (figs. 1. d and e).

Total nitrogen uptake by crop was found significant by all the treatments except mono-inoculation with R_{56}. Out of two groups (mono and co-inoculation) the average N uptake was 92 and 120 kg ha^{-1} respectively and it was 30 % more by co-inoculation. Its correlation (R^2=0.754) with oven dried weight of nodules was significant (fig. 1. f).

All the individual isolates (whether *Rhizobium* or PGPR) were found non-significant over FUI towards total P uptake by crop while co-inoculation found to increase the P uptake significantly except combination of R_{40}+P_3 and R_{56}+P_3. It was 21 % higher by co-inoculation group over mono-inoculation group (Table 3).

With regard to total potassium uptake by crop only three isolates R_{40}, P_3 and P_{10} could increase it significantly over FUI while all the isolates in different combinations were found significantly superior. Average total potassium uptake by crop due to mono-inoculation group was 38 kg ha^{-1} while with co-inoculation group it was 41 kg ha^{-1} with a increase of 8% (Table 3). Rudresh *et al*., (2005) [44] stated that the combined inoculation of organisms increased nutrient uptake by chickpea as compared to either individual inoculations or an uninoculated control. It appeared that *Rhizobium* inoculation enhances nitrogen fixation while an important action mechanism of PGPRs is root growth pattern modification caused by growth regulators such as auxins and gibberellins (Gutierrez Mañero *et al*., 2001 [24]; Probanza *et al*., 2002 [41]). The auxins enhanced the useful root surface in contact with the soil, thus, increasing nutrient absorption.

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 crops considering grain and straw yields, nitrogen uptake by grain and the total nitrogen uptake by the crop (grain + straw) over FUI respectively (Table 1 and 3). 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. Park, (1988) [36] reported that parameters like biological yield and harvest index are closely related to sink size, source activity and sink source ratio. Olsen, (1982) [35] reported that photosynthesis, dark reaction and the partitioning of assimilates are the essential prerequisite for increased and stable plant productivity. Malik *et al*., (2006) [32] 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. Although BNF is a natural process, many soils do not have sufficient numbers of appropriate rhizobia for effective symbiosis. Inoculating legume crops with compatible rhizobia ensures maximal BNF. (Silva and Uchida, 2000 [45]; Dobbelaere *et al*., 2003 [16]; Zhang *et al*., 1997 [57]). The N_2 fixation process is energy demanding, consuming about 16ATP synthesis from orthophosphate absorbed from the soils to fix one molecule of N_2 (Giller, 2001) [19]. 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 (De Freitas *et al*., 1993 [14]; Zhang *et al*., 1996 [56]; Dashti *et al*., 1998 [13]). Groppa *et al*. (1998) [22] suggested that co-inoculation leads to an increased number of the most active nodules, therefore, to a greater N_2-fixation and assimilation. Adesemoye and Kloepper (2009) [3] confirmed that PGPR such as *Bacillus amylolique faciens* and *Bacillus pumilis* can fix nitrogen and can increase plant N uptake from fertilizer *via* other mechanisms.

Post harvest soil properties

Soybean

Among different treatments no significant differences could be find with pH, EC and organic carbon but there was good appreciation in organic carbon as compared to initial one. Mono and co-inoculant's group average was also identical. Inoculation practice made positive impact towards increasing the available nutrients (NPK) and total N status of soil when compared with initial status.

Available soil nitrogen was more but non-significant due to inoculation over FUI but on considering the average of two groups i.e. mono and co-inoculation it was 235 and 240 kg ha^{-1}respectively. Similar trend was observed with soil available P but the average of individual inoculant's group and co-inoculation group was identical. This might be the result of the microorganisms involved in P solubilization, which can enhance plant growth by increasing the efficiency of biological nitrogen fixation, enhancing the availability of trace elements and by the production of plant growth promoting substances (Gyaneshwar *et al.*, 1998 [25], Athul R. Sandeep *et al.*, 2008) [6]. Available soil potassium and total nitrogen was non-significant due to inoculation over UFUI. Available soil phosphorus was 304 kg ha^{-1} due to mono inoculation and 309 kg ha^{-1} due to co-inoculation while total N was 947 and 959 kg ha^{-1} which reflected 2 % increase due to co-inoculation (Table 2).

Chickpea

After harvesting of soybean crop there was gain in soil available nitrogen to the tune of 14 kg ha^{-1} and 1kg ha^{-1} of available P in soil which was sampled before sowing of chickpea. Post harvest soil samples indicated an increase of soil organic carbon of 0.5 g kg^{-1} soil as compared to post harvest soil samples of soybean.

Co-inoculation of R_{40} with P_3, P_{10}, P_{25}; R_{56} with P_{10} and R_{58} with P_3 could increase the available soil nitrogen significantly over FUI. Considering the two groups of inoculation i.e. mono and co-inoculation the average available nitrogen was 245 and 267 kg ha^{-1} respectively which indicate an increase of 22 kg ha^{-1} (9%) due to co-inoculation over mono-inoculation practice. Available soil phosphorus did not indicate any significant contribution over FUI and the average status between two inoculation group was also found same i.e. 21 kg ha^{-1}. The available P status of the soil improved by the addition of *P. striata* with *Rhizobium spp*. and AM fungus (Zaidi et. al. 2003) [55]. With regard to available K and total soil N no significant changes were observed against FUI by different isolates either individually or in combination but the co-inoculation was found to increase the available P by 7 kg ha^{-1} and total nitrogen by 15 kg ha^{-1} over mono inoculation (Table 4).

Table 1: Effect of mono and co-inoculation with *Rhizobium* and 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	Germination (%) at 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_{27}	68±5.2a	31±3.0b	0.22±0.02a	2482±106b	5147±212b	272±14c	15.5±0.2b	174±12.0c	32.53±0.27b	43.55±0.5a	96±14.0a
R_{33}	66±3.8a	29±6.3b	0.24±0.02b	2552±102b	5404±351b	280±15c	15.8±0.7b	181±11.9c	32.20±1.21b	45.84±2.0a	104±15.3a
R_{35}	70±5.0a	31±3.3b	0.29±0.03b	2483±132b	5286±568b	273±24c	15.8±0.7b	169±11.7c	32.25±1.23b	47.48±1.2a	97±24.0a
P_3	64±5.9a	25±4.5a	0.26±0.04b	2660±100b	5561±409b	276±16c	16.3±1.0b	191±10.6c	32.50±0.91b	46.55±2.3a	101±15.8a
P_{10}	72±5.7a	28±2.7b	0.28±0.03b	2750±122b	5538±288b	297±19c	16.9±1.1b	192±13.1c	33.20±0.32c	46.78±1.8a	121±19.4a
P_{25}	70±6.0a	30±3.1b	0.25±0.02b	2500±95b	4962±178b	258±19c	15.8±1.3b	172±10.4c	33.50±0.06c	47.33±1.8a	82±19.5a
$R_{27}+P_3$	65±5.3a	27±3.1b	0.22±0.02a	2720±125b	5405±253b	335±10d	18.2±0.6c	187±10.5c	33.47±0.05c	47.74±3.4a	159±9.7b
$R_{27}+P_{10}$	78±3.8b	23±1.4a	0.24±0.03b	2761±105b	6216±244c	364±23d	18.8±0.9c	206±5.4d	30.78±0.90a	46.05±1.4a	189±23.0c
$R_{27}+P_{25}$	74±6.8a	33±1.4b	0.31±0.02b	2690±120b	5431±287b	316±21c	18.1±1.1c	184±11.8c	33.15±0.30c	47.50±2.1a	140±20.6b
$R_{33}+P_3$	86±3.8b	28±2.6b	0.27±0.01b	2660±124b	5367±306b	304±17c	17.2±0.9b	182±6.4c	33.18±0.31c	47.61±1.1a	128±17.4a
$R_{33}+P_{10}$	82±6.8b	24±1.5a	0.30±0.01b	2778±47b	5852±309c	379±17e	19.7±1.1c	196±8.1c	32.28±0.86b	49.81±2.8b	204±16.5d
$R_{33}+P_{25}$	76±8.3b	24±3.9a	0.29±0.04b	2764±69b	5649±126b	336±16d	19.2±0.7c	195±9.7c	32.85±0.35b	46.76±1.3a	161±15.6b
$R_{35}+P_3$	70±7.1a	31±3.2b	0.28±0.06b	2697±121b	6017±191c	338±15d	18.0±0.8b	209±10.8d	30.93±0.87b	46.37±3.3a	162±14.7b
$R_{35}+P_{10}$	85±3.6b	24±1.4a	0.29±0.02b	2860±50c	6175±239c	386±14e	20.7±0.8d	213±5.1d	31.70±0.56b	47.92±1.2a	211±17.2c
$R_{35}+P_{25}$	75±9.1b	27±2.2b	0.31±0.04b	2810±66c	5656±56b	340±12d	18.4±0.7c	193±2.9c	33.18±0.31c	50.53±2.3b	165±12.0b
FUI	60±4.0a	20±1.2a	0.15±0.04a	2139±91a	4941±417b	176±11b	12.1±0.6a	135±11.6b	30.41±1.06a	44.33±1.2a	00
UFUI	58±6.0a	18±1.7a	0.14±0.03a	1897±99a	3794±199a	120±9a	9.8±0.7a	103±5.1a	28.78±0.75a	44.10±0.6a	-55
SED	8.40	4.31	0.04	148.03	419.37	24.00	1.25	14.06	1.00	2.58	24.05
LSD (p=0.05)	16.97	8.72	0.08	299.17	847.54	48.60	2.52	28.41	2.03	5.21	48.60
C.V. (%)	16.57	22.94	23.05	8.05	10.91	11.45	10.48	10.96	4.38	7.79	28.01

Table 2: Effect of mono and co-inoculation with *Rhizobium* and PGPR isolates on soil chemical properties after harvest of soybean crop.

Treatments	pH	EC	OC	Available nutrients (kg ha^{-1})			Total N (kg ha^{-1})
				N	P	K	
R_{27}	7.14±0.02a	0.22±0.01a	3.4±0.01b	233±3.3a	20.5±0.7b	306±8b	920±11a
R_{33}	7.20±0.02b	0.21±0.01a	4.5±0.03e	230±3.8a	21.0±1.5b	315±10b	942±8a
R_{35}	7.19±0.01b	0.27±0.02c	3.1±0.03a	232±5.5a	21.8±2.4b	308±9b	964±14b
P_3	7.12±0.01a	0.20±0.02a	4.3±0.02e	248±6.4b	18.8±1.4b	281±9a	959±16b
P_{10}	7.24±0.01c	0.22±0.02a	4.1±0.03d	227±6.2a	17.0±1.3a	303±10b	945±14a
P_{25}	7.10±0.01a	0.23±0.01b	3.6±0.06b	237±7.0a	18.8±1.1b	313±12b	954±8b
$R_{27}+P_3$	7.13±0.01a	0.19±0.01a	4.6±0.11f	247±5.8b	17.7±0.6a	313±6b	965±11b
$R_{27}+P_{10}$	7.14±0.01a	0.20±0.01a	3.4±0.02b	246±3.9b	20.5±2.5b	309±10b	954±12b
$R_{27}+P_{25}$	7.13±0.01a	0.19±0.01a	3.7±0.12c	244±6.3b	18.3±1.7b	300±6b	961±16b
$R_{33}+P_3$	7.14±0.01a	0.20±0.01a	3.3±0.03a	238±2.1b	16.5±1.0a	304±9b	969±8b
$R_{33}+P_{10}$	7.21±0.01b	0.20±0.01a	4.0±0.06d	239±4.0b	18.8±1.1b	315±13b	959±11b
$R_{33}+P_{25}$	7.17±0.01b	0.23±0.01b	4.0±0.01d	235±5.7a	18.5±1.3b	318±5b	937±9a
$R_{35}+P_3$	7.20±0.02b	0.21±0.01a	3.9±0.08c	246±5.2b	17.0±1.1a	311±8b	967±7b
$R_{35}+P_{10}$	7.22±0.01c	0.21±0.01a	4.0±0.20d	236±7.6a	17.3±0.9a	310±11b	973±12b
$R_{35}+P_{25}$	7.13±0.01a	0.20±0.01a	4.3±0.01e	228±12.6a	18.7±0.8b	301±11b	943±6a
FUI	7.13±0.01a	0.20±0.01a	4.1±0.03d	221±10.7a	15.3±0.8a	281±9a	967±6b
UFUI	7.12±0.01a	0.19±0.01a	3.6±0.06b	219±4.8a	14.0±0.9a	263±11a	947±7a
SED	0.02	0.02	0.10	9.19	1.95	13.33	15.46
LSD (p=0.05)	0.04	0.03	0.20	18.57	3.94	26.94	31.25
C.V. (%)	0.35	10.69	3.60	5.51	15.10	6.22	2.29

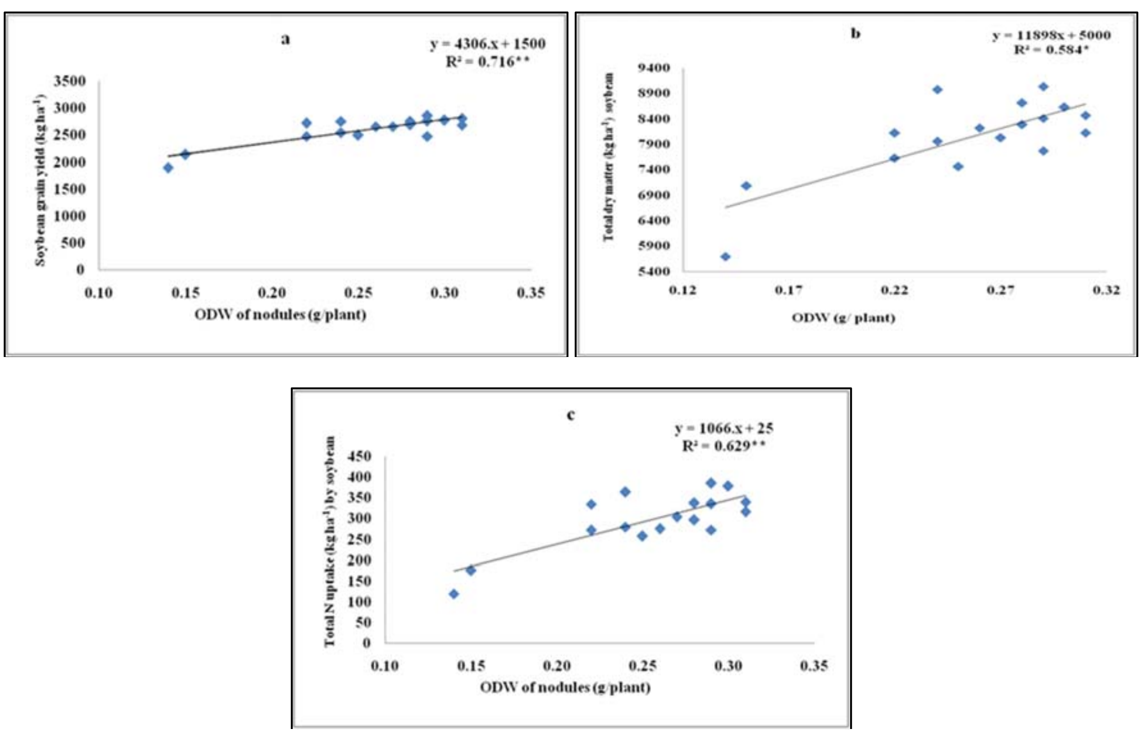
Table 3: Effect of mono and co-inoculation with Rhizobium and PGPR isolates on germination, nodulation, grain and straw yield and total NPK uptake by chickpea crop, harvest and nitrogen harvest index and additional BNF.

Treatments	Germination (%) at 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_{40}	58±7.8b	18±1.2a	0.11±0.009a	2232±41c	4127±29b	94±3.9c	14.0±0.7b	38±0.4c	35.10±0.51b	72.46±2.7a	18±3.9a
R_{56}	60±3.5b	15±2.3a	0.16±0.006c	2128±46b	4073±37b	88±3.1b	13.7±0.6a	37±0.4b	34.31±0.55a	70.26±2.7a	11±3.1a
R_{58}	56±6.9b	15±1.7a	0.12±0.007b	2122±8b	4139±53b	90±1.4c	14.6±0.3b	38±0.6c	33.89±0.23a	71.55±2.7a	13±1.4a

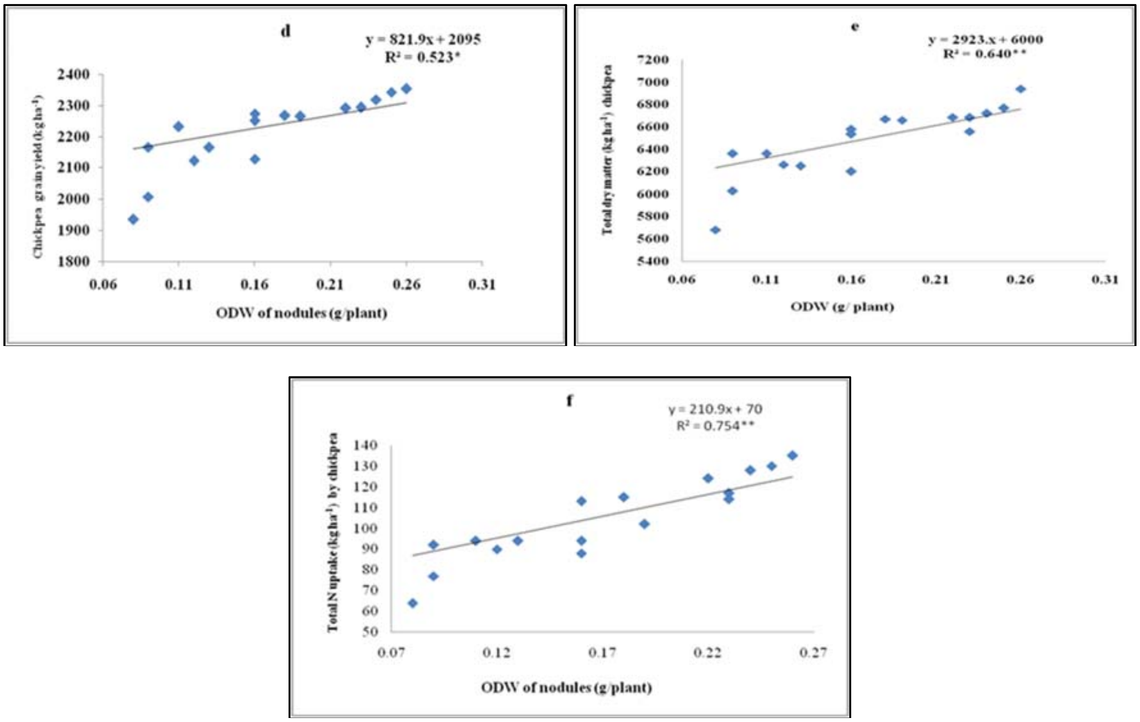
P_3	55±8.4a	14±1.3a	0.13±0.015b	2165±20b	4084±32b	94±2.2c	14.0±0.7b	38±0.4c	34.65±0.24a	72.80±2.2a	18±2.2a
P_{10}	68±3.2b	22±2.1b	0.16±0.020c	2274±22c	4262±23c	94±5.0c	15.1±1.0b	40±0.8d	34.79±0.15b	68.49±3.6a	17±5.0a
P_{25}	51±3.1a	20±1.0b	0.09±0.008a	2167±21b	4194±40c	92±2.7c	13.0±0.8a	37±0.5b	34.07±0.40a	69.31±1.0a	16±2.7a
$R_{40}+P_3$	60±3.5b	17±3.5a	0.19±0.013c	2266±31c	4395±33d	102±4.6c	15.4±0.6b	40±0.5d	34.02±0.43a	69.11±2.0a	26±4.6b
$R_{40}+P_{10}$	54±4.3a	15±1.5a	0.26±0.005e	2354±32d	4588±86e	135±5.3e	17.9±0.9c	43±0.2e	33.92±0.59a	71.37±0.7a	59±5.3d
$R_{40}+P_{25}$	60±5.4b	19±3.1a	0.23±0.014d	2295±46c	4389±47d	114±4.5d	16.7±1.5b	41±0.4d	34.34±0.69a	71.16±3.0a	38±4.5b
$R_{56}+P_3$	56±5.5b	16±3.2a	0.16±0.019c	2252±50c	4327±19c	113±6.0d	15.3±1.4b	39±0.5c	34.22±0.51a	74.03±2.9a	37±6.0b
$R_{56}+P_{10}$	56±5.5b	16±2.5a	0.24±0.014d	2320±55c	4401±57d	128±1.7e	17.2±1.4b	40±0.2d	34.52±0.80a	72.83±0.6a	52±1.7c
$R_{56}+P_{25}$	54±5.5a	19±3.6a	0.22±0.008d	2292±37c	4394±60d	124±6.6d	16.9±1.2b	40±1.0d	34.29±0.61a	74.63±0.8b	47±6.6c
$R_{58}+P_3$	60±7.9b	13±2.8a	0.23±0.007d	2294±18c	4265±73c	117±3.2d	20.1±3.3c	40±0.9d	34.99±0.54b	74.68±1.3b	41±3.2c
$R_{58}+P_{10}$	61±9.2b	13±2.7a	0.25±0.008d	2344±48d	4426±30d	130±7.2e	17.4±0.4b	41±0.8d	34.61±0.60a	72.41±1.8a	53±7.2c
$R_{58}+P_{25}$	60±5.4b	16±2.7a	0.18±0.019c	2268±15c	4403±42d	115±6.5d	17.0±1.4b	41±0.7d	34.00±0.32a	73.97±1.3a	38±6.5b
FUI	51±2.4a	15±2.0a	0.09±0.007a	2008±35a	4022±28b	77±5.8b	13.2±0.8a	36±0.4b	33.30±0.25a	73.16±1.0a	0
UFUI	40±4.6a	13±1.9a	0.08±0.007a	1936±37a	3746±57a	64±4.1a	10.5±0.3a	32±0.7a	34.07±0.28a	72.71±1.9a	-12
SED	7.87	3.28	0.02	51.56	67.45	6.35	1.70	0.80	0.71	2.78	6.35
LSD (p=0.05)	15.91	6.63	0.03	104.20	136.31	12.84	3.43	1.61	1.43	5.62	12.84
C.V. (%)	19.71	28.64	4.34	3.29	2.24	8.62	15.58	2.89	2.91	5.46	32.40

Table 4: Effect of mono and co-inoculation with *Rhizobium* and PGPR isolates on soil chemical properties after harvest of chickpea crop.

Treatments	pH	EC	OC	Available nutrients (kg ha^{-1})			Total N (kg ha^{-1})
				N	P	K	
R_{40}	7.18±0.01b	0.24±0.01a	5.1±0.11d	237±3.6a	21.0±1.3b	304±9c	956±8.2a
R_{56}	7.21±0.01b	0.22±0.01a	5.6±0.12e	239±7.2a	23.0±1.6c	313±9c	958±6.1a
R_{58}	7.17±0.01a	0.35±0.01c	4.1±0.06a	237±8.6a	22.0±2.2b	306±8c	980±9.1a
P_3	7.22±0.01c	0.22±0.01a	4.6±0.04c	249±7.0a	21.0±1.5b	278±10b	975±8.4a
P_{10}	7.15±0.01a	0.25±0.01a	4.2±0.03b	257±14.1a	19.0±1.1b	300±12c	961±5.4a
P_{25}	7.24±0.02c	0.25±0.01a	4.4±0.10b	252±7.4a	21.0±1.2b	311±11c	970±6.1a
$R_{40}+P_3$	7.19±0.01b	0.28±0.02b	4.2±0.04b	268±7.7b	21.0±0.9b	313±7c	1003±19.5a
$R_{40}+P_{10}$	7.23±0.01c	0.24±0.02a	4.1±0.04a	272±10.7b	23.0±1.7c	309±9c	973±7.5a
$R_{40}+P_{25}$	7.24±0.01c	0.29±0.02b	4.3±0.04b	274±7.7b	21.0±1.2b	300±5c	979±8.0a
$R_{56}+P_3$	7.15±0.01a	0.26±0.01a	4.2±0.04b	266±4.3b	19.0±1.1b	304±9c	1008±11.9a
$R_{56}+P_{10}$	7.18±0.01b	0.24±0.00a	4.2±0.04b	267±10.3b	22.0±1.6b	315±13c	978±10.3a
$R_{56}+P_{25}$	7.16±0.01a	0.29±0.02b	4.0±0.03a	263±5.1b	21.0±1.5b	319±6c	956±12.5a
$R_{58}+P_3$	7.16±0.01a	0.26±0.01a	4.3±0.21b	273±6.3b	20.0±1.6b	311±7c	986±10.4a
$R_{58}+P_{10}$	7.19±0.01b	0.28±0.02b	4.0±0.05a	263±7.7b	20.0±0.8b	311±15c	992±10.8a
$R_{58}+P_{25}$	7.17±0.01a	0.33±0.03c	4.6±0.04c	259±4.4b	22.0±1.6b	302±7c	962±8.5a
FUI	7.15±0.01a	0.29±0.02b	4.0±0.04a	239±5.7a	16.0±0.5a	273±9b	978±11.9a
UFUI	7.14±0.01a	0.23±0.01a	3.9±0.05a	236±5.9a	14.0±0.7a	248±10a	958±8.2a
SED	0.01	0.02	0.11	11.12	1.95	12.17	14.31
LSD (p=0.05)	0.03	0.04	0.22	22.47	3.94	24.60	NS
C.V. (%)	0.25	11.25	3.59	6.14	13.56	5.72	4.25



Soybean



Chickpea

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

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