Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(1): 1591-1593
Received: 06-11-2017
Accepted: 07-12-2017

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Study on the Effects of PSB and Rhizobium with their combinations on nutrient concentration and uptake of chickpea (*Cicer arietinum* L.)

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Abstract
This experiment was carried out to determine the effects of PSB and rhizobium inoculation for a Chickpea (*Cicer arietinum* L.) variety, Awarodhi, under Central Uttar Pradesh conditions in 2015 and 2016. The trial was laid out in factorial randomized block design with three replications. The experiment on the chickpea (*Cicer arietinum* L.) crop was conducted at Nawabganj Research Farm of Chandra Shekhar Azad University of Agriculture and Technology, Kanpur (U.P.) during Rabi season for year 2015-16, to assess the response of Rhizobium and PSB as affected by their application. The result shows that some plant attributes of the crop like the number of nodules varied from 8 to 25 at 30 days and 12 to 43 at 60 days. The yield of the crop was also varied from 12 to 26 qtl. ha^{-1} of seeds of chickpea (*Cicer arietinum* L.) and 13.7 to 35.0 qtl. ha^{-1} Stover of the crop. Maximum N and P uptake in grain and straw as well as protein content in grain were noted maximum with dual inoculation of (Rhizobium + PSB).The treatment combination Rhizobium + PSB has been found best in respect of number nodules, seed and stover yield, nitrogen, phosphorus content and its uptake and also in protein content of seed.

Keywords: Rhizobium, *Cicer arietinum* L.

Introduction
Pulse crops have a specific importance for the vegetarian population of our country because pulses are the major source of protein. Pulses are important component to sustain the agriculture production as the pulse crops possess wide adaptability to fit into various cropping systems, improve the soil fertility and physical health of soil while making soil more porous due to tap root system (Verma, 2016) [15]. India is the largest producer and consumer of pulses. Gram (*Cicer arietinum* L.) is occupying third position among the grain legumes in the world. Among the pulses, gram occupies a predominant position and is considered as a "King of pulses". It is originated in south eastern Turkey. Chickpea (*Cicer arietinum* L.) is grown in 54 countries with nearly 90% of its area covered in developing countries. The major chickpea(*Cicer arietinum* L.) producing countries are India (64%), Pakistan (8%), Turkey (6%), Iran (3%), Myanmar (3%), Australia (3%), Ethiopia (3%), Canada (2%), Mexico (2%), Iraq(1%), and others (5%) in 2004/05 to 2006/07 (FAOSTAT, 2012). The number of Chickpea (*Cicer arietinum* L.) importing countries has increased from 30 to 150 during 1981 to 2011. Chickpea (*Cicer arietinum* L.) reached a record high global area of 13.3 million ha and production of 11.75 million tons during 2011. In 2013 the area of Chickpea (*Cicer arietinum* L.) cultivation increased to 13.5 m ha but production remained at 13.1 million tons (FAOSTAT, 2015). Chickpea (*Cicer arietinum* L.) contains on the average 22% protein, 4.5% fat, 63% carbohydrate, 8.0% crude fiber and 2.7% ash (Miao *et al*., 2009). Yield and yield components analysis provides a framework for identifying potentially useful traits for yield improvement (Kobree *et al*. 2010) [8]. The escalating price of fertilizers in recent years, limit their use in crop production. Therefore, the nutrient requisition through chemical fertilizers, if supplemented with low expense natural sources will not only economize the nutrient use but also improve the soil health and factor productivity on sustainable basis (Verma *et al*., 2016) [15]. Grain yield of Chickpea (*Cicer arietinum* L.) was increased significantly with *Rhizobium* and phosphorus application (Raut and Kohire, 1991) [9]. These bacteria, through biological nitrogen fixation, meet about 80%–90% of the total N requirements of legumes (Verma 1993) [14]. Likewise, phosphate-solubilizing bacteria (Biological Phosphate Fertilizer (BPF) have the capability to solubilize the residual or fixed soil P (Singh *et al*. 2008) [10]. Chickpea (*Cicer arietinum* L.) is more efficient than other pulses in taking up P from soil, as it secrets more acid which helps in solubilizing P.

The combined inoculation of Rhizobium and phosphate solubilizing bacteria has increased nodulation, growth and yield parameters in Chickpea (*Cicer arietinum* L.) (Alagawadi and Gaur, 1988; Gupta and Namdeo, 1997; Jain *et al*., 1999; Khurana and Sharma, 2000) [1, 5, 7]. The combined inoculation of Rhizobium and Phosphate Solubilizing Bacteria has increased nodulation, growth and yield parameters in Chickpea (*Cicer arietinum* L.).

Materials and Methods

The pot experiment was conducted on chickpea (*Cicer arietinum* L.) during the winter season of 2015–2016under natural conditions at Nawabganj Research Farm of Chandra Shekhar Azad University of Agriculture and Technology, Kanpur. The soil of the experimental field was alluvial in origin. Surface soil sample (0-15 cm.) depths were initially drawn from randomly selected parts of the field before sowing. The quantity of soil sample was reduced to about one kilogram through quartering technique. The soil sample was then subjected to mechanical and chemical analysis in order to determine the textural class and fertility statusthe soils were sampled to a depth of 0-30 cm of the soil, air-dried and sieved (2 mm) for soil analyses. Some physicaland chemical properties of soils are given in Table 1.

Table 1: Some properties of the <2 mm fraction of the top 30 cm of soil used for the site.

Sr. No.	Particulars	Values
1.	Sand (%)	43.05
2.	Silt (%)	33.74
3.	Clay (%)	23.20
4.	Textural Class	Loam
5.	pH	7.80
6.	Electrical Conductivity (dS m^{-1})	0.80
7.	Organic Carbon (%)	0.42
8.	CEC[C mol(P^{+})kg^{-1}]	13.24
9.	Available Nitrogen (kg ha^{-1})	181.24
10.	Available Phosphorus (kg ha^{-1})	11.08
11.	Available Potassium (kg ha^{-1})	171.04
12.	Available Sulphur (kg ha^{-1})	12.86
13.	Available Zinc (kg ha^{-1})	0.48
14.	Bulk Density (Mg m^{-3})	1.40
15.	Particle Density (Mg m^{-3})	2.64
16.	Porosity (%)	47.00

The sample so drawn was subjected to physical and chemical analysis using standard methods. Each treatment was replicatedthrice. The treatment having 16 combinations with inoculation of Rhizobium and Phosphate Solubilizing Bacteria (PSB).The experimental soil was sandy loam with pH (1:2) 7.80, EC (1:2) 0.80dS m^{-1}, andavailable N, P, and K of 181.24, 11.08 and 171.04 kg ha^{-1} of soil, respectively. Chickpea (*Cicer arietinum* L.) variety namely, AWARODHI ('Desi' type; small seeded) matures in 100-120 days was taken.The treatment consisted of three promising Rhizobium and PSB inoculants which were obtained from CSA Uni. Microbiology laboratory. Each plot had gross plot size of 1 m × 1 m (1 m^2). Seeds were planted 30 cm between rows and 10 cm between plants. The inoculation process was done as based on the recommended procedure. Before planting, inoculation of the seeds was done using the dish as a container and sugar as adhesive material to stick the inoculums on the seeds. Before planting, inoculation of the seeds was done. Inoculants (6.25 g kg^{-1} of seed) was applied according to the treatments before and at the time of sowing, respectively. Plant analysis was done by harvesting the samples before flowering means at pre flowering stage and then at pod setting stage and harvesting stages of crop growth were first air dried under shade, and then kept in an oven at 70^{O}C for 12 hours to become free from moisture. The oven dried samples were ground, passed through 60 mesh sieve and stored in the sample bottles. For N analysis in plants Kjeldhal' s methodhave been used and for P analysis calorimeter. Nitrogen content in grains was determined by Kjeldhal's method and then protein content was obtained by multiplying the nitrogen content with the factor 6.25.Statistical analyses of the data were carried out according to factorial randomized block design. All the parameters weresubjected to analysis of variance (ANOVA), using afactorial procedure.

Results

The effects of inoculation was clearly seen on different parameters of growth and yield of Chickpea (*Cicer arietinum* L.) plant. Inoculation of seed with Rhizobium + PSB produce significantly higher number of nodules in comparison to other inoculants. The number of nodules ranged from 8 to 25 after 30 days of sowing and 12 to 43 after 60 days of sowing. It is obvious from the data that treatment of seed with different inoculants have increased the seed yield from 12.00 to 26.20 qtl. ha^{-1} as well as yield of stover was also increased considerably from 13.70 to 35.00 qtl.ha^{-1}. On an average, the highest nitrogen content in seeds (3.51% to 3.70%) is recorded with Rhizobium+ PSB and lowest was obtained through uninoculated plots. In stover the highest nitrogen content (1.02% to 1.07%) is recorded which is slightly high.The highest protein content was recorded in Rhizobium+PSB i.e. 23.03% and lowest i.e. 22.48% with uninoculated treatment which is due to higher yield of nitrogen in seed of Chickpea (*Cicer arietinum* L.). The nitrogen uptake in seeds significantly increased from 42.12kgha^{-1} to 96.94 kgha^{-1}while in stover the uptake 13.92kg ha^{-1} to 37.45kg ha^{-1}. Phosphorus uptake in seeds of Chickpea (*Cicer arietinum* L.) differs from 7.20 kg ha^{-1} to 17.29kg ha^{-1} while in stover uptake changes from 7.29kg ha^{-1} of uninoculated plot to 8.79kg ha^{-1} in inoculated plots. The application of microbial inoculants (Rhizobium + PSB) significantly increased the yield and protein content in grain and straw of chickpea (*Cicer arietinum* L.) genotypes. The results in Table 2 itself describes the validity of the treatment, it clearly signifies the effect of interaction of rhizobium and PSB is very much helpful in acquiring better production and productivity of the crop.

Discussion

The data on the effect of different inoculants and different levels of phosphorus on nodules numbers, seeds and stover yield, content and uptake of nitrogen and phosphorus in grains as well as in stover and protein content in both grain and stover has been described. The data pertaining to the effects of different inoculants and their interactions at 30 and 60 days have been. It is evident from the data that different inoculants differed significantly in respect to their effect on nodule number. (Bandyoupadhay, 2002) [2] observed that nodulation, growth and seed yield were augmented significantly when the enrichment of the soil was made with naive rhizobia from where they has been isolated.The inoculation of efficient PSB isolates significantly increase the plant height and biomass than control (G. Dakshayini *et al*. 2016) [3]. The Rhizobium + PSB recorded highest number of nodules followed by Rhizobium, PSB and uninoculated treatmentdifferent inoculants increased seed and stover yield significantly in

comparison to uninoculated treatments. The different inoculants also differed statistically in respect to their effect on seed and stover yields. It has been observed that increase in seed and stover yields were higher in magnitude with mixed inoculants. On an average, the highest seed and stover yields were recorded with Rhizobium + PSB inoculation. The content and uptake of nitrogen and phosphorus both in seeds and stover were shown a significantly positive effect due to different inoculants senhanced (G.S. Tagore *et al.* 2014) [12]. Undoubtly increase in nitrogen availability in soil leads to increase in content of nitrogen and phosphorus application in seed and stover of chickpea (*Cicer arietinum* L.).

Table 2: Effects of different inoculants treatment on different growth parameters of Chickpea (*Cicer arietinum* L.)

Sr. No.	Treatments	No. of nodules at 30 days of sowing	No. of nodules at 60 days of sowing	Seed Yield (qtl. ha^{-1})	Stover Yield (qtl. ha^{-1})	Nitrogen Content in seed (%)	Protein Content in seed (%)	Nitrogen uptake in seed (kg ha-1)	Nitrogen uptake in Stover (kg ha-1)	Phosphorus uptake in seed (kg ha-1)	Phosphorus uptake in stover (kg ha-1)
1.	Without Inoculation	9.00	15.75	13.99	15.7	3.51	22.48	50.38	16.06	8.57	3.81
2.	Inoculation with Rhizobium (Rh)	15.00	24.00	16.30	20.4	3.65	22.55	59.55	21.15	10.22	5.27
3.	Inoculation with PSB	13.25	22.25	16.10	20.0	3.63	22.70	58.59	20.64	9.89	5.25
4.	Inoculation with Rh+ PSB	17.25	36.75	19.98	28.0	3.68	23.03	76.34	29.40	12.96	7.25

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