Journal of Plant Development Sciences

Meerut, India

RESEARCH

EXPLORATION OF SOIL PROPERTIES AND GROWTH OF CHICKPEA, GROWN WITH SOIL MICROBIAL INOCULANT FOR SUSTAINABLE AGRICULTURE

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Received-01.03.2025, Revised-16.03.2025, Accepted-30.03.2025

Abstract: In this study, we examine the effects of *Rhizobium* and Phosphate Solubilizing Bacterium (PSB) on the attributes of the soil and the growth of *Cicer arietinum* L. (Chickpea). In this experimental work, the *Rhizobium* and PSB were used individually and in two combined inoculations. We measured various morphological, physiological and biochemical parameters using standard protocols. The findings of this field experiment showed that the test crop's biomass, plant height (42.6 %), grain yield (50 %), plant nitrogen (35.14 %), phosphorus, and carbon content (24.62 %), as well as the plant protein content (49.56 %), all had significantly increased (20-40%) by the dual inoculated plant. The soil's nutrient content was also improved. The data obtained from this experimental work indicates that the crops inoculated with a combined application of *Rhizobium* and PSB had higher yield and growth than single inoculants. So their combined application can be a better substitute for chemical fertilizers.

Keywords: *Rhizobium*, PSB, Dual inoculation, *Cicer arietinum*

INTRODUCTION

Chickpea (*Cicer arietinum* L.), an important Rabi season legume crop that originated from South-Eastern Turkey is as mall herbaceous annual bushy plant that has a maturation period of 90 days. The plant with a height of 20-50cm branches profusely with primary, secondary and tertiary kinds. Its stems are erect, solid, hairy and green in color with pinnate leaves comprising 5-7 pairs of leaflets with toothed edges having both kinds of glandular and non-glandular hairs on its surface. Flowers are solitary, bisexual, and self-pollinated are borne on an axillary raceme of white, pink, purple or blue color. Their pods are small, roundish, swollen with hairy structures possessing a pointed beak with 1-2 seeds per pod with round or irregular in shape, wrinkled or smooth surfaces of creamy or brownish color. During 2021- 22, chickpea production in India was 13.75 million tonnes from an acreage of 10.91 million ha with productivity of 12.6 q/ha (DES 2023, MoAF &W, GoI).

Nutritious seeds as the most important edible part of the plant are high in protein, carbohydrates and fibers are a good source of iron, zinc, magnesium, phosphorus, folic acid and vitamins (B6 and B9) [USDA, 2018]. They are also rich in calcium, iron, and niacin.

Chickpea as a member of the family Fabaceae (Leguminosae) is widely accepted for crop rotation practice due to its nature to fix atmospheric nitrogen by symbiotic association with *Rhizobium* sp. This in turn helps in enriching the soil nutrients for succeeding crops. For sustainable agriculture practices, there is a challenging and perspective requirement of eco-friendly nitrogen fertilizers such as biological nitrogen fixation which converts atmospheric nitrogen into a usable form for the plant with the help of microbial processes. These microbes, found in nodules, are formed on the primary and secondary roots as the site of the nitrogen fixation.

Chemical fertilizers are in extensive use from the very past to increase the yield of crops but in return they deplete the soil fertility and harm the microflora of the soil. Biofertilizers, as a good substitute of these chemical fertilizers, being environmentally friendly and renewable sources of nutrients can fulfill the demand of plant nutrient uptake to maintain soil health and fertility.

Biological Nitrogen Fixation (BNF) is a natural process of reduction of molecular nitrogen into ammonia with the help of microorganisms. The nitrogen-fixing bacteria such as *Rhizobium* converts the atmospheric nitrogen into usable form with the help of the enzyme nitrogenase. This is done by them by forming root nodules specifically confined to the leguminous plants and triggering plant growth.

Phosphate Solubilizing Bacterium (PSB) solubilizes the phosphate by the action of organic acids synthesized by microorganisms and helps in the growth of the plant [Abbasi *et al.*, 2015]. The combined inoculation of *Rhizobium* and PSB (R + PSB) has been reported to be more effective than their sole application. Plants of chickpeas exhibit

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enhancing growth, nodulation, leghemoglobin content, and yield.

The present study was designed to evaluate the performance of single and dual inoculation of *Rhizobium* and PSB on the growth, nodulation and yield of chickpeas and some physico-chemical characteristics of the soil.

MATERIALS AND METHODS

Exploratory Site and Design

The experiment was designed in four equivalent plots: two plots for the sole treatment of *Rhizobium* and PSB, one plot for dual inoculation of both of them; and one for the control.

Material Used

• Certified seeds of Chickpea (*Cicer arietinum*) and

• *Rhizobium leguminosarum* and Phosphate Solubilizing Bacteria (*Bacillus megaterium*) for the treatment of chickpea were procured from the Microbiology Division of IARI, New Delhi.

Seeds Inoculation

Seeds of chickpea were treated with 0.5g *Rhizobium* and 0.5g PSB individually and in the combination of both. A 10 %jaggery solution was prepared. This jaggery solution acts as a cement to stick the chosen biofertilizers uniformly on the seed coat of the seeds. Seeds were immersed into the solution until the development of an even layer of biofertilizers. After that, the seeds were dried in the shade and then sown into the field, according to the experimental design.

Soil Parameters

pH

The concentration of hydrogen ions in the soil referred toas its pH was measured after the germination of seeds. One gram of soil was blended in 10mL of DW and the pH of soil suspension was recorded with the help of Systronics micro pH system 361.

cation exchange capacity

It was used as the holding capacity of the cations by the soil particles. These cations are held by the anions through electrostatic force and are adequately known as exchangeable with various cations therefore, they are accessible for plants.

For CEC the accompanying equation was utilized:

(pH of test soil - pH of 1N acetic acid) x 22 m.eq/100g soil test

Where the pH of 1N acetic acid = 2.3

soil bulk density

It is known as the mass of dry soil per unit of mass volume including the air spaces. Higher organic matter in the soil lowers the bulk density whereas the compaction of the soil particles increases its bulk density. It was determined as follows:

$$\text{Bulk Density } (\text{g/cm}^3) = \frac{\text{Soil Weight (g)}}{\text{Soil Volume}(\text{cm}^3)}$$

particle density

Particle Density of soil as the mass of individual soil molecule/unit volume was determined by the given equation:

$$\text{Particle Density } (D_p) = D_w \cdot W_s$$

$$W_s = W_{sw} - W_w$$

porosity

It incorporates the number of pores or open spaces between soil particles. Pore spaces are raised by the development of roots, worms, and creepy crawlies. Soil porosity was calculated with the help of the given formula:

$$\text{Soil Porosity } (\%) = \left[1 - \left(\frac{\text{Bulk Density}}{\text{Particle Density}}\right)\right] \times 100$$

Seed Germination

Developinga new plant from the seed is referred toas seed germination. It was measured as follows:

$$\frac{\text{Number of Germinated Seeds}}{\text{Number of Seeds Sown}} \times 100$$

Physiological Parameters

Some important attributes such as plant length, biomass and yield were recorded after harvesting the crop. The length of the root and shoot were estimated independently with the assistance of measuring scale (cm). The entire plant length was the sum of the length of root and shoot. The fresh and dry weights of the plant, pods plant^{-1}, seeds pod^{-1}and 100 seeds weight were recorded.

Biochemical Parameters

chlorophyll content

It was calculated as:

$$\text{chl a (mg/g tissue)} = \frac{12.7\,(A_{663}) - 2.65\,(A_{645}) \times V}{1000 \times W}$$

$$\text{chl b (mg/g tissue)} = \frac{22.9\,(A_{645}) - 4.68\,(A_{663}) \times V}{1000 \times W}$$

$$\text{Total chl (mg/g tissue)} = \frac{20.2\,(A_{645}) - 8.02\,(A_{663}) \times V}{1000 \times W}$$

Where;

V = Chlorophyll extract final volume

A = Absorbance at a particular wavelength

W = Fresh weight of tissue

protein and proline content

Amino acid chains make up proteins. In addition to being a crucial macronutrient, it is a significant part of every cell. Hormones, enzymes, membrane channels, and pumps all develop from protein. Proline is a cyclic -amino acid that serves as an antioxidant and osmolyte. They were determined as:

$$\frac{\text{OD} \times \text{Factor} \times \text{Dilution (assuming any)} \times 1000}{100 \times \text{Total Volume}}$$

nitrate reductase

Nitrate reductase activity was calculated by the following equation:

$$\frac{\text{OD} \times \text{Factor} \times \text{Dilution (assuming any)} \times 1000}{100 \times \text{Total Volume}}$$

nutrient content

Some chemical nutrients of soil and plants examined were nitrogen, phosphorus and natural carbon. The total nitrogen (%) was computed by the following formula:

$$\frac{14 \times \text{Normality of acid} \times \text{Actual titrant volume} \times 100}{\text{Sample weight} \times 1000}$$

The deduction of phosphorus and organic carbon was separately determined by the given equation:

$$\frac{\text{OD} \times \text{Factor} \times \text{Dilution (assuming any)} \times 1000}{100 \times \text{Total Volume}}$$

Statistical Analysis

The data collected from the observations obtained with the use of biofertilizers on chickpeas were analyzed statistically. For this, the single-way ANOVA was performed by utilizing the IBM SPSS 16.0 statistical tool.

RESULTS AND DISCUSSION

While evaluating the impact of biofertilizers on soil and plants, the effect due to individual and combined use varied as per the attributes studied. The attributes affected included such as pH, CEC, bulk density, particle density, porosity of soil, carbon, nitrogen and phosphorus content of soil and plant, seed germination percentage, plant morphological, biochemical and yield characters.

Soil attributes

The attributes affected were included such as pH, CEC, bulk density, particle density, porosity and soil nutrients (C, N, P). The pH of the soil of control was slightly alkaline (8.45), but on inoculation of PSB alone, *Rhizobium* alone and the combined inoculation was neutral pH (7.36). The acidity and alkalinity of the soil are determined by its ph. Chickpea crops are grown in slightly alkaline soil. The above results make it possible to assert that the microorganisms have the ability to maintain the pH at an optimal level. Findings are consistent with Jangir *et al*., 2017.

The highest CEC concentration (11.22) was observed in the dual-inoculated soil. It avails of the nutrients of soil to the plants' uptake, so it has a positive impact on soil fertility. As the CEC increases soil nutrient conditions improve (Jangir *et al*., 2017). Cation exchange capacity as the measurement of the soil's ability to hold positively charged ions influences pH, structure, stability, nutrient availability, and how the soil responds to fertilizers and other ameliorants. The soil that had been treated with *Rhizobium* + PSB had the highest cation exchange capacity against CEC levels in untreated soil. The pH of the soil has a direct impact on CEC. Maximum CEC was recorded at the optimal pH, and CEC values decreased with an increase in pH. The CEC makes soil nutrients available to plants, resulting in a positive effect on soil fertility. However, the effects of these biological fertilizers are insignificant as the pH rises and the CEC values fall. Similar results have also been recorded earlier by (Jangir *et al*., 2017).

Bulk density and porosity responded alike. Particle density is inversely related to bulk density and porosity. The maximum particle density was found in control (3.83) and minimum with dual inoculation followed by *Rhizobium*(2.41, 3.10 serially) (Fig. 2). The differences in PSB and *Rhizobium* inoculation on bulk density, particle density, and porosity are not appreciably different. However, the highest bulk density and porosity were seen in plants that had received both PSB and *Rhizobium* inoculation. Particle density found as inversely related to bulk density produces an effect that is the reverse of that of these treatments. Therefore, the soil treated with a dual inoculation of *Rhizobium* + PSB showed the lowest particle density against untreated soil found with the highest particle density.

Soil Nutrients

Phosphorus, Carbon and Nitrogen contents were found maximum (0.13, 0.084, 0.39 systematically) in dual inoculated soil followed by the individual treatments i.e., *Rhizobium* (0.09, 0.077, 0.34) and PSB (0.11, 0.072, 0.28) (Fig. 3). Biofertilizers improved soil fertility by converting inaccessible supplies of elemental nitrogen, bound phosphorus, and non-exchangeable potassium into soluble forms that plants could absorb. Heisnam *et al.,* (2017) reported similar outcomes. The synergistic effects of *Rhizobium*, PSB, and PGPR on nitrogen fixation and the solubilization of native soil phosphorus boosted the availability and absorption of these nutrients by the crop plant Pandey *et al.,* (2015). This improves the soil's nutritional status (Heisnam *et al*., 2017). The enhanced carbon content could result from the *Rhizobium* and PSB treatment of the seeds, responsible for the boosting of the activity of bacteria that in turn can break down the inaccessible carbon and provide it to the plants. In comparison to the control, *Rhizobium* and PSB significantly increased the organic carbon content (Nissa *et al*., 2017; Rajaa and Takankhar, 2017). The solubilization and mineralization of N and P content got increased all the chosen parameters in plants as well.

Seed Germination

Seed germination percentage of chickpea showed the stimulatory effect of selected microorganisms. The individual inoculation of *Rhizobium* and PSB had a stimulatory effect on morpho-physiological and biochemical parameters. A higher value of germination was found in the plots treated with dual inoculation of PSB and *Rhizobium* (Table1). The capacity of the inoculants to inhibit the growth of antagonists existing in soil and by releasing chemicals that promote plant growth around seeds sowed contribute to an increase in germination percentage. The combination of inoculation of phosphorus-solubilizing bacteria and salt-tolerant *Rhizobium* is reported to improve chickpea germination (Pawar *et al*., 2018).

Plant length

Dual inoculation of *Rhizobium* and phosphate solubilizing bacterium significantly increased plant height (84.13cm) over their sole application and control. Plant nutrients as naturally present in the soil are mostly present in forms that plants cannot use. Nitrogen and phosphorus are the nutrients that have the most controlling impact on the growth and output of plants. The provision of nutrients for plant development and crop production is largely made possible by soil microorganisms. Co-inoculation of microorganisms is more successful than single inoculation. The combination inoculation of *Rhizobium* and PSB boosted plant height in chickpea. The findings of Pawar *et al*., (2018) and the current results are all in consistence.

Yield Attributes

Yield characteristics of chickpea as plant biomass (fresh as well as dry), pod no. /plant, seeds/pod, seed weight increased maximum (49.39, 14.56, 35.67, 1.60, 5.27 respectively) in the plants treated with dual inoculation of PSB and *Rhizobium* followed by *Rhizobium* and PSB single inoculants (Table2). Plant biomass is a crucial characteristic to be considered when evaluating the effects of any treatment. The treatment of *Rhizobium* and PSB alone, as well as their co-inoculation, had a stimulatory impact on the chosen legume crops. By making more soil nitrogen and phosphorus available to crops, *Rhizobium* and PSB are proven to be advantageous. The biological nitrogen-fixing activity of *Rhizobium* in leguminous crops is well recognized. A significantly higher yield was obtained with the *Rhizobium* and PSB inoculation of application of in treatment T8 (*Rhizobium* + PSB + DAP). The grain yield increased by 39.5% in T8 (*Rhizobium* + PSB + DAP) over control (T1) (Katiyar *et al*., 2020). The usage of biofertilizers in the placement of chemical fertilizers or conjunction with chemical fertilizers has expanded over the past few years because of their advantageous function and non-harmful behavior towards nature. The productivity and metabolism of plants get increased by PSB and *Rhizobium*, which in turn help plants absorb more phosphorus and nitrogen. This results in better plant health. Such results are also on earlier records (Abid *et al*., 2016; Rasool and Singh, 2016; Pawar *et al*., 2018).

Biochemical parameters

Biochemical parameters such as chlorophyll, protein, proline contents, nitrate reductase activity and nutrients (C, N, P) also responded similarly and gradually increased in the dual inoculated plants (Fig. 1). Similar studies were shown by Samar and Kumar, 2020.

Chlorophyll content

When *Rhizobium* sp. was co-inoculated with PSB, there were no significant variations in chlorophyll concentration when compared to *Rhizobium* sp. alone. The increased availability of N and its absorption by a greater root surface area accompanied by much more root hairs and lateral root growth can be the cause of the stimulatory effect on chlorophyll concentration. Rhizobacteria increase photosynthetic activity and nutrient absorption, prolong leaf senescence, and benefit photosynthesis (Kaur *et al*., 2015).

Protein and proline

The combined action of these biofertilizers enhances the protein content of leguminous crops while their single application of these fertilizers stimulates protein synthesis. Identical outcomes were noted by Diep *et al.* (2016). The proline content of leguminous crops is negatively correlated with their growth characteristics. Plants make proline, a cyclic amino acid when they are under stress. Thus, a higher level of proline directly reflects plants that are under stress. The findings of the present study are consistent with those of (Ziyaul *et al*., 2017). Microorganisms break down organic molecules, aiding in the absorption of carbon by plants. As more chlorophyll enhances a plant's ability to photosynthesize, more carbon is used and accumulates as photosynthate, which is a sugar, increasing the amount of protein and chlorophyll in a plant indirectly consequently increasing carbon absorption.

Plant nutrients

Nitrogen as essentially required for the synthesis of amino acids is demanded by plants when making seeds as an appropriate time when proteins are formed. Phosphorus is well recognized to play a role in the metabolism of carbohydrates, and it also serves as an energy carrier from metabolism and is stored as phosphate molecules for later use in plant growth, development, and production. Chickpea yield increased when PSB and *Rhizobium* were used together because of their synergistic effects. This could be because there is more nitrogen and phosphorus available to plants, which consequently leads to better nutrient uptake. The present findings support earlier observations made by (Rasool and Singh, 2016; Heisnam *et al.*, 2017; Pawar *et al.*, 2018).

The Nitrate reductase activity

The Nitrate reductase activity is positively impacted by both dual inoculation and solitary application. Individual applications have some reports but combined applications are not so much recorded for study. According to Singh et al., (2015) and Singh *et al.*, (2018), PSB has a stimulatory impact on the activity of the nitrate reductase and nitrogenase enzymes in lentils. *Rhizobium* treatment has also been reported with a similar effect (Crusciol *et al.*, 2018).

Phosphate solubilizing bacterium and *Rhizobium* improve plant growth by various mechanisms such as alteration in the composition of rhizosphere microorganisms, production of plant signaling compounds, bacteriocins, siderophores, plant growth hormones and improving the availability of nutrients by rhizosphere microorganisms. Similar results were observed by (Diep et al., 2016; Pawar *et al.*, 2018; Samar and Kumar, 2020).

Table 1. Percent germination in treated chickpea after 8, 16, 24 and 63 days of sowing (DYS).

TREATMENT	% GERMINATION			
	8 DYS	**16 DYS**	**24 DYS**	**63 DYS**
Control	22	68	72	84
Rhizobium	40	90	92	98
PSB	50	88	88	94
R+PSB	48	92	92	98

Table 2. Biomass and Yield attributes affected by different combinations of biofertilizers (*Rhizobium*, PSB & R+PSB).

Treatment	Length (cm)		Dry weight (g)		Pods/Plant	Seeds/Pod	Wt. of 100 Seeds/Plant (g)
	Shoot	**Root**	**Shoot**	**Root**			
Control	45.83	13.17	2.27	0.22	8.333	1.067	355.00
Rhizobium	62.33	15.17	5.39	0.45	13.333	1.467	392.00
PSB	52.00	14.50	3.98	0.36	13.333	1.400	375.33
R+PSB	66.63	17.17	13.74	0.82	35.667	1.600	526.67

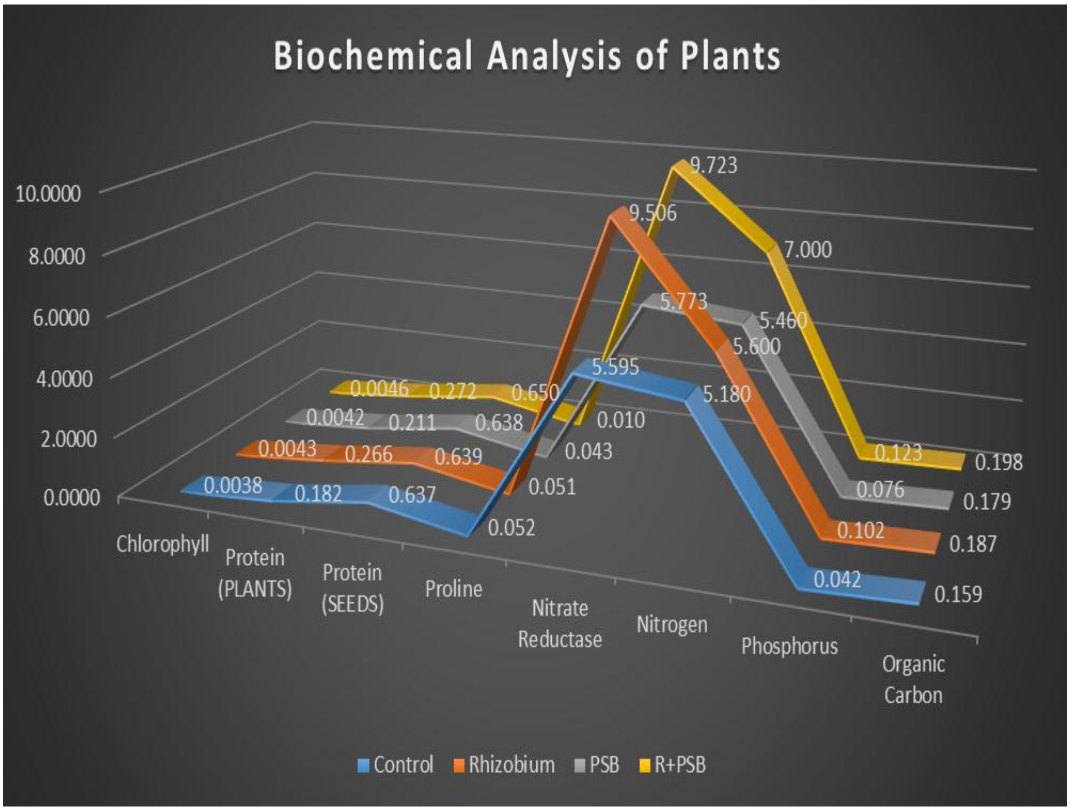

Fig.1: Biochemical analysis of chickpea plant when treated

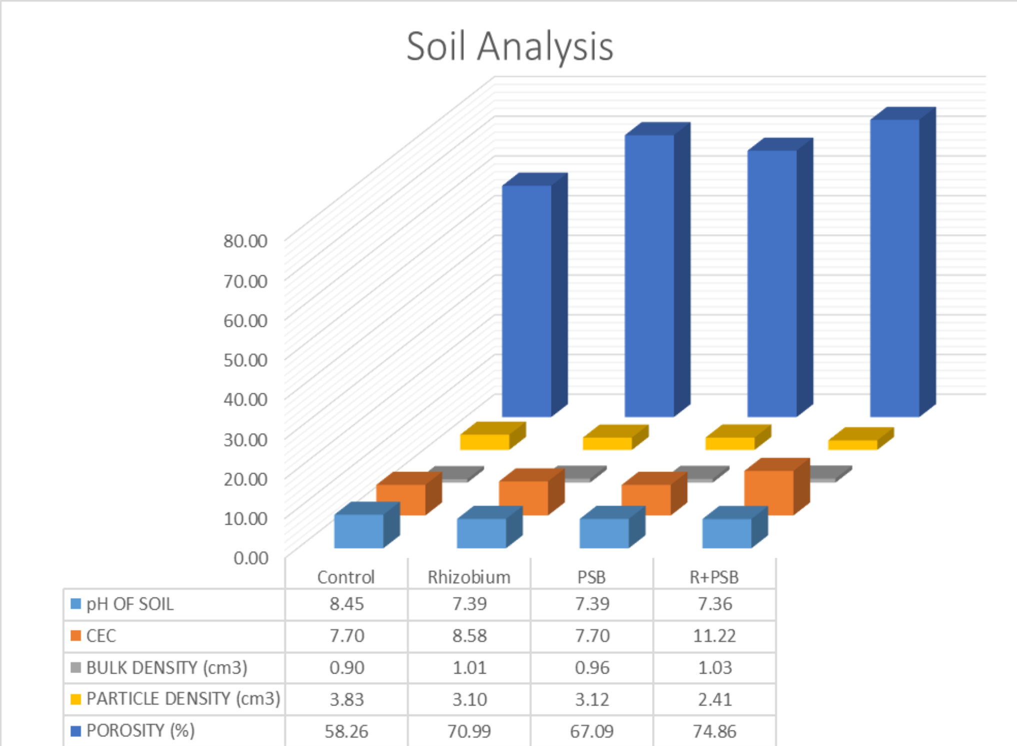

Fig.2: Analysis of soil treated with different combinations with different biofertilizers.of biofertilizers (*Rhizobium*, PSB & R+PSB).

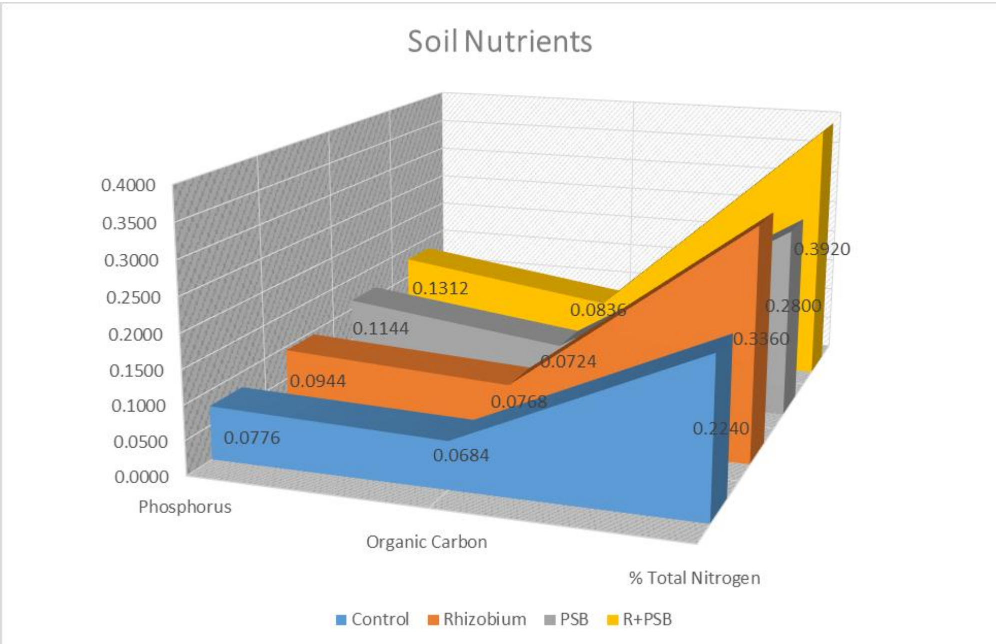

Fig.3: Impact of different combinations of biofertilizers on nutrient content of soil.

CONCLUSION

The results of the current study supported by statistical analysis showed that the inoculation of PSB and *Rhizobium* together had a positive favorable impact on chickpea growth and yield. The results further supported the hypothesis that, in comparison to individual *Rhizobium* or PSB treatments, the dual inoculation method enhances the biomass and nutritional content (N, P, and C) of chickpea. However, with the microbial inoculation treatment, the physiological characteristics of the soil did not greatly alter.

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