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Effect of Rhizobacterial strains inoculation with organo – minerals fertilizer on the soil properties and yield attributes of Chickpea (Cicer aritenum)

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ABSTRACT: The low organic matter, soil poor structure and extensive cropping pattern are the main characteristics of Pakistan's agriculture. In the current study the goals were set to sustain soil quality and productivity can only be achieved through sustainable management practices. Plant growth promoting rhizobacteria (PGPR) are important soil microbes enchant growth and yield of plants through direct and indirect mechanisms of root colonization or seed inoculations. Five bacterial strains isolated from rhizospheres of Chickpea (*Cicer aritenum.*L*)*, Lentil (*Lens culinaris*) Barseem (*Trifolium alexendrium*), mungbean (*Vigna radiate*) and Sesame (*Sesamum indicum*) were morphologically and biochemically screened and identified. Field experiment was performed at the Arid Zone Research Center, Dera Ismail Khan (Pakistan). These five bacterial strains were used as augmentation material with chickpea seeds. The results showed that treated plots with inoculation of rhizobium strains showed highest shoot and root weights (37.66, 7.00 g). Maximum root length (50 cm) was found in treatment T14 (*Rhizobium ciceri*) along with organic amendments as compared to control treatment. In arid environment, the effect of rhizobial strains (PGPR) on chickpea showed that significantly increases the crop germination percentage. This combination thus increases the uptake of nitrogen and phosphorus in treated plots of rhizobacteria strains T14 treated chickpea crop. The research revealed that the use of inoculation treatments plots have significantly higher yield than non-treated. Treatment with *Rhizobium ciceri* along with compost, which showed higher grain yield (8 %) as compared to the control. Therefore, it may be recommended for the farmers to use organic manures along with mineral fertilizers, synthetically active organic products to sustain production and saving soil.

Keywords: **Rhizobacteria, organic amendments, chickpea growth & yield.**

I. INTRODUCTION

Chickpea (*Cicer arietinum* L.)

Chickpea (*Cicer* arietinum L) is the most important staple legume in various developing countries like Pakistan. It has great importance in terms of nutritional value for human and animal populations. Chickpea is a winter season crop primary grown in rainfall areas of Pakistan. Chickpea is valued for its nutritive seeds with high protein content. Chickpea contains protein content (16 to 20%), carbohydrates (51%), fat (25.8%), total dietary fiber, vitamins and minerals (Hirdyani, 2014). Pakistan is second largest chickpea growing country and also third highest production producing country about 561 kg/ha^{-1} (FAO, 2014).Legumes has significance due its nitrogen fixation characteristics. Pulses in cropping system can increase the symbiotic N fixation and improve phosphorus level of soil as well (Sinclair, Vadez, 2012). Developing a symbiotic relationship with host plants, rhizobacteria plays an important role, especially in legumes. Atmospheric nitrogen, fixation N_2 that is converted by *Rhizobium leguminosarum* in legume crops which contributes half of 175 million tons of gross organic nitrogen fixation per year around the world. The approximate amounts N fixed in legumes crops contributes up to 176 kg N ha^{-1} per year depending on the cultivar, bacterial strain and natural components. (Gopalakrishnan et al. 2014).

Organic fertilization

Organic fertilization derived from animal matter, compost and crop residue for improving organic matter protection of top-soil of field.(Soares et al. 2013) claim that the usage of agricultural waste such as substrate chances an ancient essential for either add profit to this kind of waste after degrading the price of production of seedlings. organic fertilizers increase the chemical and biological, physical, structure, also improve the soil organic matter The larger amount of organic matter rises soil structure, allows water and nutrient absorption, decrease erosion as well rise plant growth. organic fertilizer consequences in prompt increase in soil organic carbon, which is generally connected to the degree of carbon applied (Chantigny et al. 1999).

Plant growth promoting rhizobacteria are significant microorganisms that can be found in the rhizosphere, in root surfaces and in relationship with roots, which can enhance the nature or extent of plant growth effectively and significantly. Vast area of bacteria including types of, Alcaligenes, *Arthrobacter, Burkholderia,Pseudomonas*, *Azospirillum, Azotobacter*, Klebsiella, Enterobacter Bacillus and Serratia have revealed as PGPR to improve plant growth (Kloepper et al., 1989; Okon and Labandera-Gonzalez, 1994; Glick, 1995).

Plant growth promoting rhizobacteria are group of microorganism which not just enhance plant growth but also put sigficant impact on soil. Yet in adding accepting to enhance the crop yield in record of controlling diverse pollutions and pathogen (Ahmad and Kibret, 2013). Soil nutrients availability and fertility of the soil (Glick, 2012). For the most part, PGPR are mainly playing role in plant growth through essential mineral nutrient directing levels of plant hormone, bio controlling various plant pathogens. As the rhizobacteria are utilized worldwide by agronomist with goal to make the crop profitable. Also, a comprehensive research is going on worldwide with more important inspiration to uncover the new attributes of these bacteria (Ma et al*.,* 2011; Wani & Khan, 2010) The two-third N2 is evaluated universally through organic nitrogen fixation (BNF) while the rest is changed over by mechanically with help of (Haber–Bosch process) (Rubio & Ludden. (2008).

II. MATERIALS AND METHODS

A field study was conceded out in winter time (2018-19) at Arid Zone Research Center, D.I. Khan, to assess the response of rhizobacterial. Strains on Chickpea growth and yield parameters.

The field (experiment) was laid out in the (RCBD) with two factorial Randomized Complete Block Design with three replicates and six treatment. Chickpea was used as a test crop; Nifa 2005 verity was sown.

All the plots were applied N and P @:20:50 kg/ha^{-1}, respectively. Along with organic amendments (mungbean, wheat straw.lentil straw,and Compost) was applied to all the respective plots except control.

Rhizobacterial strains preparation.

The bacterial strains were collected from the Rhizosphere of Lentil, Mungbean and Chickpea field

All the bacterial strains were uprooted from Rhizosphere of lentil, Chickpea, fields. Plants samples were shifted in laboratory for further process. They were grown on the (General Purpose Media). After Inoculation, all the strains were further transfer to the (Yield Menitol Agar) YMA

Free living bacterial strains were cultured on agar plates, Maximum exo-polysaccharide production strains were selected on the basis of visual observation. After the growth of these bacteria on agar medium, they were further transferred to the broth media

After Completion of identification process of bacteria. Seed were treated in broth media. The socked seed of Chickpea in this media was cultivated in the field.

RCBD

Chickpea verity – Nifa 2005

Treatment Detail

Main Plots organic amendments were as follows:

S_1: Control

S_2: Mungbean straw

S_3: Compost

S_4: Wheat straw

The bacterial Strains were as follows:

T_1: Control

T_2: *Enterobacter asburiae*

T_3*: Enterobacter mori*

T_4*: Rhizobium ceceri*

T_5: *Pseudomonas aeruginosa*

T_6*: Pseudomonas putida*

Statistical Study.

The data obtained during the study was subjected to statistical analyzed by the software Statistix 9.1. The analysis of variance and least significance difference for comparison of means was measured as per procedure given by Steel et al. (1997).

III. RESULT AND DISCUSSION

Plant height. (cm)

The application of organic amendment along with PGPR Inoculation positively increase the growth parameters and yield of chickpea sown. (Table No.1). The Maximum plant height (36.000 cm) was observed with Treatment T4 (*Rhizobium ceceri* along with Mungbean straw) which showed 5.67% increase over control. And the lowest plant height was recorded in T1 control.(24.33) The interaction of organic amendments and inoculation along with chemical fertilizer was also showed significant results during the experiment. The maximum Plant height was (35.00) and the least value was recorded in T1 control (24.00). The results are in line with (Zahran. 2011).The colonization of root associated beneficial microbes in the rhizosphere is important for their beneficial effect on plant growth.

Nodule Count

Nodule counting was significantly affected in the presence of organic amendments along with rhizobacterial strains and wheat straw, and compost. (Table No 1). Nodule counting Inoculation along with organic amendment caused a maximum increase in Nodule counting as compared to control. The maximum value was (44.6) found in Treatment T4 (*Rhizobium ceceri* along with wheat straw) and the least value was found in control T1 (21.66) as reported by (Ahmad et al., 2014) this type of association between legume and Rhizobium is well documented and also confirmed its significant role nodule formation in different legumes.

No of pods per plant^{-1}

No of Pods per Plant^{-1} was significantly affected in the presence of inoculation and organic amendments.(Table No.1) The No of Pods per plant was significantly affected by Inoculation along with organic amendment caused a greater rise in No of Pods per plant as compared to control. The maximum value was (44.667) found in Treatment T4 (*Rhizobium ceceri* along with Wheat straw) and least value was found in T1 (Control +mungbean straw) (23.33) (Fatima et al. 2008).Suggested that PGPR inoculation the Number of Pods of Chickpea with rhizobia increased plant growth, dry matter, number of pods.

Root length (cm)

Root Length was significantly affected in the presence of inoculation and organic amendments.(Table No. 1). The Root length was significantly affected by organic amendment and inoculation. The maximum value was T6 (*Pseudomonas putida* along with Compost) (52.66) and the least value was found in T1 (Control along with Mungbean straw) (25.00) These results are supported by the work of Ahmad et al. (2011) where they described that co-inoculation of PGPR and rhizobia increased the root length and improved water uptake from deeper soils.

Plant fresh and dry weight (g)

Plant fresh weight was significantly affected by Inoculation along with organic amendment caused a greater increase plant fresh weight as compared to control.(Table No.2) The maximum value was (37.33) found in Treatment T2 (*Enterobacter asburiae* along with Wheat straw) Followed by T4 and the least value was found in T1 (Control along withCompost) (17.66) Plant dry weight was significantly affected by Inoculation along with organic amendment caused a greater increase in Plant dry weight as compared to control. The maximum value was (9.00) found in Treatment T4 (*Rhizobium ceceri* + Wheat straw) and the minimum value was found in T1 (Control plus Compost) (6.00) Similar finding was observed by (Ben Romdhane et al.2009). That Soil inoculation positively improved, shoot dry weight in the semi-arid Saharan region.

Grain yield kg/plot

Data reveled that Grain yield of chickpea was significantly affected by organic amendment along with inoculation during the experiment Grain yield was significantly increase 39 percent the maximum value was recorded in Treatment T4 (*Rhizobium ceceri along with* Wheat straw) (179.67) and followed by T2 (*Enterobacter asburiae along with* Mungbean straw), T3 (*Enterobacter mori*), T6 (*Pseudomonas putida)*, and T5 (*Pseudomonas aeruginosa*) and the least value was recorded in T1 (Control along with wheat straw) as reported by (Ahmad et al., 2014)This type of association between legume and Rhizobium is well documented and also confirmed its significant role on yield and nodule formation in different legumes.

Figure.1. Soil Nitrogen as affected by PGPR Inoculation along with organic amendments

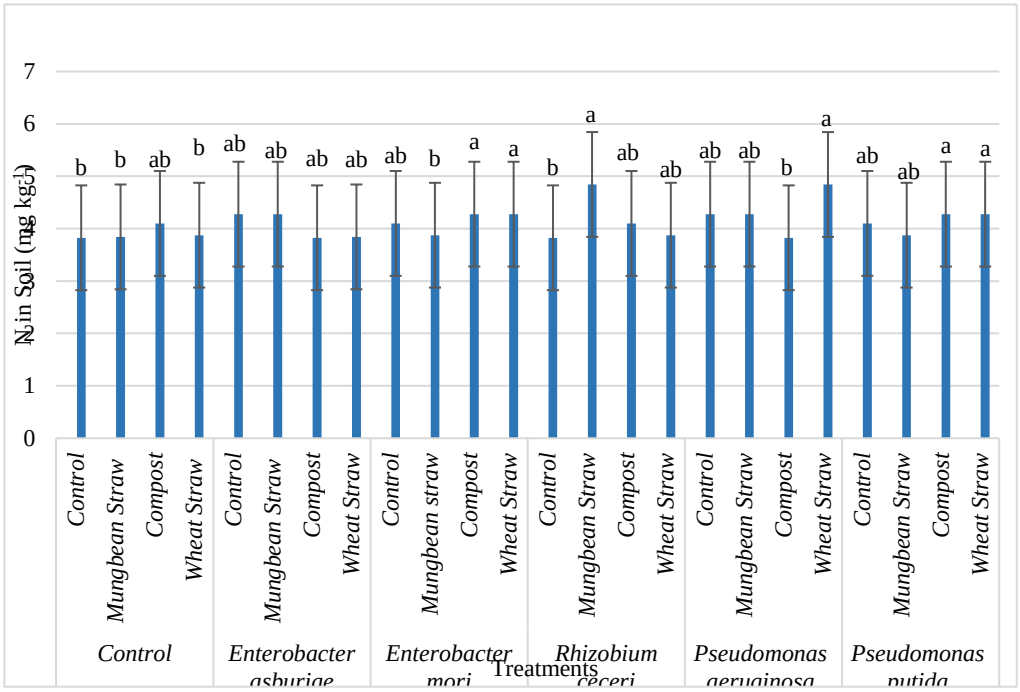
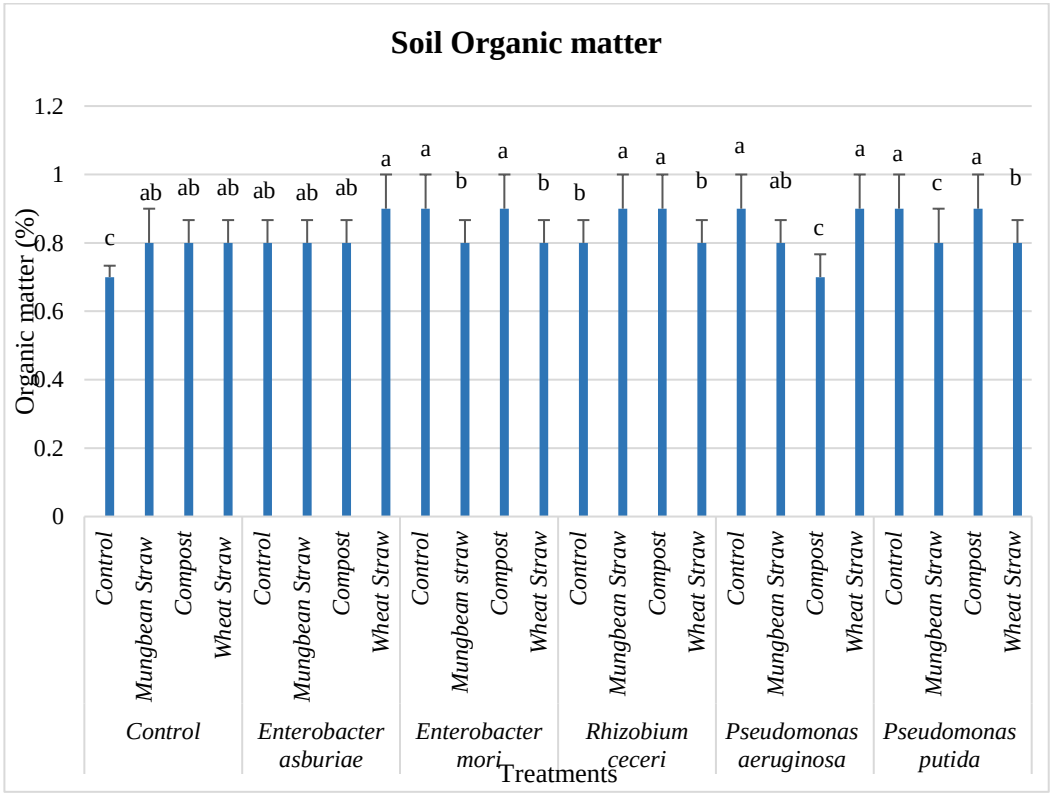


Figure.2. Soil Organic matter as affected by PGPR Inoculation along with organic amendments



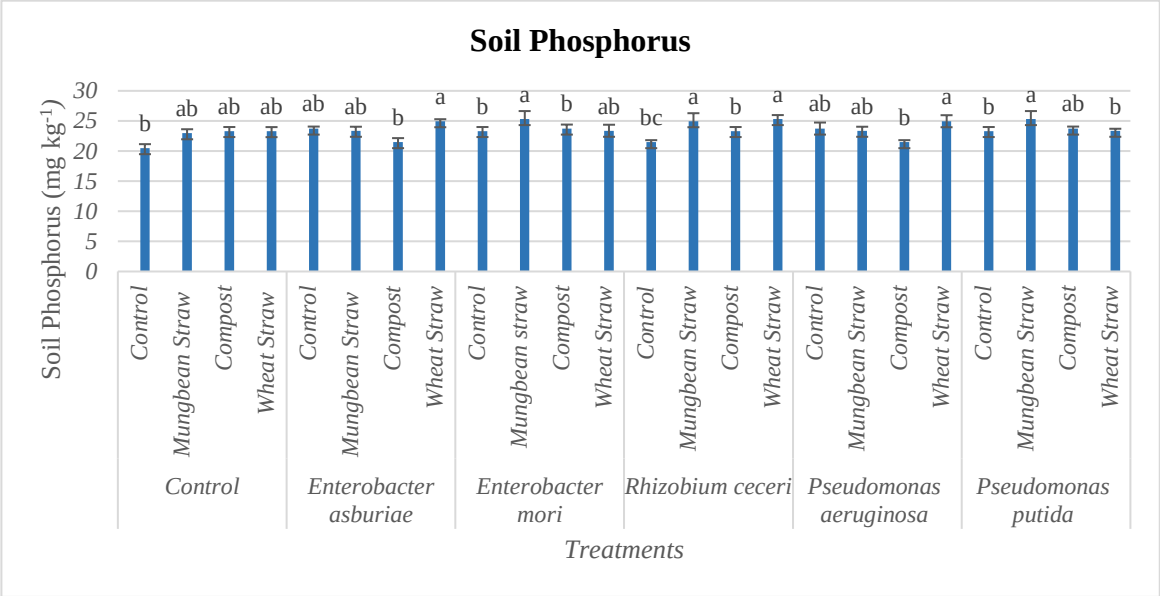


Figure.3. Soil Phosphorus as affected by PGPR Inoculation along with organic amendments

IV. Soil available Nitrogen Phosphorus and Organic matter %

Bio fertilizers plus organic amendment treatments caused a significant increase in soil available nitrogen, phosphorus and organic matter percentage during the experiment. (Figure No.1)Phosphorus content in soil was significantly increase in seed treated with inoculation treatments over control. The maximum phosphorus content in soil was recorded in treatment 4 while the lowest value was recorded in T1Control.The results are in line with (Verma et al. 2010).PGPR have been shown to solubilize precipitated Phosphates and enhance phosphate availability to chickpea that represent a possible mechanism of plant growth promotion under field condition. (Figure No. 2) Nitrogen content of the soil is also significantly changed by the inoculants. Similarly the greatest value was recorded in the treatment T4, while the lowest in the treatment T1 Control. (Ma et al. 2011) reported that the rhizobacteria increases the soil nitrogen content up to some extent. (Gamalero et al. 2004) found that the N content becomes improved due to the PGPR inoculation.(Figure No.3) Data shows that inoculant showed positive response to increase the soil organic matter because the values are significantly changed where the greatest was observed in the treatment T4 while the lowest were recorded in the treatment control. The results are in line with (Shahbaz et al. 2014).suggested that the application of bio fertilizer showed that they increase organic matter and essential plant nutrients to the soil. Also that PGPR can enhance and improve the availability of soil phosphorus due to the phosphate solubilizing. The results showed that crop residue and PGPR Inoculation improve soil potassium. Data shows that inoculant also play a vital role on the soil organic matter because the values are significantly changed where the greatest was observed in the treatment T1 while the lowest were recorded in the treatment control. The results are in line with (Shahbaz et al. 2014). The application of bio fertilizer to the soil adds up which increase organic matter and essential plant nutrients to the soil.

V. Conclusion

Microbes plays important role in improving yield of Chickpea. The use of PGPR (*Rhizobium ciceri* along with compost) strains were most effective in enhancing plant growth parameters thus rhizobacterial strains showed positive response on soil properties. The other isolated strains and inoculants have shown that there have been an optimization in shoot and root parameter of chickpea. As a result, various inoculants used while the most effective strains was *Rhizobium ciceri* along with compost. It may be recommended that *Rhizobium ciceri* has the potential to increase the nutrients of soil while also optimizing the agronomic parameters of the Chickpea crop.

TABLE NO.1.Effect of crop residue along with PGPR inoculation on plant height no of pods root length, nodule count and grain yield.

Treatments	Plant height (cm)				No of pods per plant^{-1}				Root length (cm)				Nodule count				Grain yield kg/ per plot			
	Control	Control	Mungbean straw	Compost	Wheat straw	Mungbean straw	Compost	Wheat straw	Control	Mungbean straw	Compost	Wheat straw	Compost	Mungbean straw	Control	Wheat straw	Control	Mungbean straw	Compost	Wheat straw
T1	25.667 cd	30.667 ch	23.333 gh	31.333 cd	34.000 bc	28.333 ab	24.000 d	28.667 ab	33.333 de	25.333 g	32.333 ef	29.333 fg	21.667 ij	20.000 j	23.000 hj	27.333 ei	143.07 ab	130.67 de	158.67 ab	129.00 de
T2	27.000 bc	41.333 ab	34.333 bc	36.333 ab	23.000 h	31.000 ab	29.000 ab	31.333 ab	43.000 ab	39.000 bc	37.667 cd	47.667 ab	31.667 cg	25.333 gj	27.000 fi	35.333 abc	132.00 cd	179.00 ab	132.00 cd	127.00 de
T3	30.667 ab	33.000 bc	34.333 bc	38.333 ab	42.000 ab	30.333 ab	30.667 ab	32.000 ab	37.333 cd	38.667 bc	41.667 ab	44.000 ab	33.667 bc	23.333 hi	32.000 cf	34.000 ad	167.00 ab	164.33 ab	128.00 de	111.67 e
T4	33.667 ab	32.667 bc	24.333 fg	27.000 de	44.667 a	36.333 a	33.333 ab	34.000 ab	46.667 ab	40.000 bc	43.333 ab	41.000 bc	35.000 ad	26.667 fi	31.000 cg	38.667 ab	134.33 bc	179.67 a	127.00 de	153.33 ab
T5	27.333 bc	29.667 cd	25.333 ef	26.333 ef	40.333 ab	30.333 ab	31.000 ab	32.000 ab	47.667 ab	42.000 ab	44.667 abc	43.667 abcd	36.000 ac	28.667 dh	32.333 bf	36.000 ac	131.33 cd	141.67 abcde	138.00 ab	176.33 ab
T6	29.333 ab	31.333 cd	24.333 fg	27.333 de	32.333 bc	31.667 ab	32.000 ab	32.000 ab	49.667 ab	43.333 ab	46.333 ab	52.667 a	32.667 bf	25.333 gj	28.667 dh	35.667 ac	129.600 de	148.00 ab	136.33 ab	141.67 ab

Mean with different letters in a column are significantly different at 5% level of significance

Table No.2. Effect of crop residue along with PGPR inoculation on plant fresh and dry weight.

Treatments	Plant dry weight (g)					Plant fresh weight (g)			
	Wheat straw	Mungbean straw	Compost	Control		Wheat straw	Mungbean straw	compost	Control
T1	8.3333 ab	7.6667 ab	6.3333 b	7.6667 ab		25.333 cd	30.667 bc	17.667 e	26.333cd
T2	8.3333 ab	8.3333 ab	7.3333 ab	7.6667 ab		22.667 de	23.667 de	34.333ab	40.333a
T3	7.0000 ab	7.6667 ab	7.6667 ab	8.6667 a		21.333 de	25.000 cd	23.667 de	21.33de

T4	7.0000 ab	7.3333 ab	7.3333 ab	9.0000 a		37.667 ab	22.000 de	25.333 cd	22.667de
T5	7.0000 ab	7.3333 ab	7.3333 ab	9.0000 a		22.667 de	21.667 de	19.667 e	25.667cd
T6	7.0000 ab	7.3333 ab	7.6667 ab	8.6667 a		20.667 e	23.667 de	21.000 e	22.333de

Mean with different letters in a column are significantly different at 5% level of significance

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