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Evaluation of bacterial strains for developing effective plant growth promoting strain on chickpea growth and physico chemical properties of soil

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The study was intended to isolate and characterize the plant growth-promoting properties. A collection of microbial consortia called plant growth-promoting microorganisms (PGPM) work to increase crop growth and yield through a variety of direct mechanisms, including as nitrogen fixation, phosphate solubilization, synthesis of PGH, ammonia, and siderophore, as well as indirect mechanisms. The aim of the study was to evaluate the impact of bio fertilizer on Chickpea growth and soil health. Five bacterial strains were utilized for this purpose. Pot study was conducted at arid zone research center D. I. Khan. The results showed that treated pots with inoculation of *rhizobium* strains plus organic amendments showed greater results as compared to control treatment. The highest value of plant height, shoot fresh and root fresh weight (34.cm)(39.66g)(6.00 g)were found in treatments T2 (*Mesorhizobium ceceri*plus compost)T6 (*Pesodomonas putida wheat straw*)as compared to control treatment. The study found that rhizobacterial strains treated plots significantly increased chickpea crop growth. Treated pots showed that using Rhizobacterial strains have increase chickpea crop growth and improve soil nutrient absorption from soil. As a result, the combination with (Rhizobacteria plus organic amendments) increases soil Nitrogen and Phosphorus content. It may be concluded from the study that using PGPR *Mesorhizobium ceceri*plus compost and *(Enterobactor mori*plusLentil straw) *increases* soil nutrient absorption form soil while also improved chickpea growth parameters.

Keywords: Organic amendments, PGPR, Growth Chickpea

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important legume crop grown worldwide. It has the nutrition values due to high in protein 25 – 28%. Content. Legumes are the second most popular group after the cereals. The developing countries contribute 74% of in the pulse production and the major pluses produce comes from developed countries. Through root nodulation in legume crop chickpea, which support biological N-fixation (BNF) by (*Mesorhizobium Cicero*plus compost)The approximate amounts N fixed in legumes crops are on average 176 kgha^{-1}, provided up to 85% N requirement in legume.(GopalaKrishnan et al. 2014).

Rhizobacteria are the group of bacteria that reside in the plant rhizosphere and contribute to in nutrient cycling, from the soil particulates to soil solution and plants (Igual et al. 2001). The function of rhizosphere microbial colonies have long been discovered, but they have become more evident in recent years for plant growth and development (Glick, 1994). The discovery and efforts were subsequently made for the production of rhizobacteria that promote plant growth by means of such rhizospheric bacteria for significant effective results. The nutrients availability and synergies with other microbes in the soil can directly be affected by the rhizobacteria. Plant growth-promoting rhizobacteria (PGPR) symbolize a wide range of soil bacteria that function together with a host plant and thus stimulate the growth of the hosts (Vessey, 2003).

Plant growth promoting rhizobacteria (PGPR) are also very important for the crop as it increase resistant towards diseases and pathogen in plants and improves crop production through the production of various organic and inorganic process in the root zone of the crop (Ahmad and Kibret, 2014). Because of the multipurpose positive effect on soil and soil properties i.e. soil bulk density, organic matter, soil fertility improvement and also decomposition of organic substances and increase nutrient uptake different forms. The use of nitrogen, phosphorus and other essential minerals as a bio-control fertilizer is used to minimize plant disease and increase crop production (Glick, 2012). Rhizobacteria are used in the world due to increase crop production and as a environmentally sustainable option. In order to increase crop production use of bio fertilizer has key role to improve plant growth and yield (Wani and Khan, 2010). The conversion of 2/3 of N_2 globally is due to biological nitrogen fixation (BNF); while there is also contribution of N factories manufacture due to the Haber-Bosch process also contributes in the plants N (Rubio and Ludden, 2008). The availability of nutrients in plants also results from the transport of soil-based nutrients by several forms of bacterial genera which are also useful for soil health (Khan et al. 2009). These bacteria can control insects and pesticides, such as use of PGPR as a bio fertilizer. (Hynes et al.2008). *Pseudomonas* is immune to the various environmentally friendly detergents, and antibiotics when apply as a bacterial strains (Raja & coll. 2006). PGPR are a biota in the soil that provides nutrient supply, improves the soil physical status and plays a positive role in solvent phosphates. Farmers and scientists are using different types of synthetic or chemical fertilizers to overcome the

Problems of soil fertility, but the use of chemical fertilizer is harmful not only to the soil, but also to the environment.

MATERIALS AND METHODS

Pot Experiment

During the year 2020, pot experiment was conducted at the Arid Zone Research Centre in Dera Ismail Khan.

Rhizoshpere sampling

Healthy plants were selected from different fields cultivated with legumes. In the respective season, (lentil, Mung bean, Barseem, Chickpea Sesame) grown at Arid Zone Research Centre (AZRC) Dera Ismail Khan (Pakistan) were uprooted while the crops were at the flowering stage.

Isolation of bacteria

Plant roots were washed in the laboratory with distilled water. Using sterilized needle, nodules were separated from roots. The separated nodules were first washed with deionized water (DI) for three minutes.Then soaked in ethanol for 30 seconds, and for 10 min in sodium hypochlorite solution 0.5%. The sterilized nodules were rinsed in sterilized DI water 3-4 times and dried with on sterilized filter paper.

Broth culture media preparation

These chemically sterilized pink and large-sized nodules were punctured with a sterilized needle and streaked for 72 hours on media plates of yeast extract mannitol agar (YMA) and incubated at 28 °C. After 72 hours, growth on Petri dish were visually observed and well-grown colonies were streaked over and over again until pure, fine and uniform colonies were obtained. Phylogenetic identification of five rhizobacterial strains was done from MicroGen® korea.

Rhizobacterial Strainsalong with organic amendments used in experiments.

(iii) Seed Inoculation

Seeds variety of desi Chickpea Nifa - 2005 was obtained from AZRC D.I.KHAN and inoculated with five rhizobacterial strains after seed Inoculation Chickpea seeds were sown in the pots at Arid Zone Research Centre, Dera Ismail Khan.

Treatment details of the experiment.

T1 control	T2 Rhizobium Ceceri plus Compost	T3 Enterobactor Asburiea Mungbean starw
T4 Enterobactor Mori Lentil straw	T5 pseudomonas aeruginosa FYM	T6 pseudomonas Putida Wheat straw

Table 1: Physico – chemical characteristics of soil prior to the experiment

Parameters	Units	Values
pH	-	7.85
EC	mSm^{-1}	0.382
Soil Total Nitrogen	%	0.04
Extractable P	$mgkg^{-1}$	5.13
Extractable K	$mgkg^{-1}$	176
Organic matter	%	0.41
Bulk density	gcm^{-3}	1.21
Textural Class	Silty Clay	

Statistical Analysis

Statistix 8.1 software was used to analyze the soil and agronomic data. For mean comparison, the Least Significant Difference (LSD) test was used at a 5% probability level (Steel, Torrie and Deekey, 1997).

Table 2: Rhizobacterial strains were identified as follows:

S.No	Plant growth promoting traits	Gram Reaction	Cultural Characteristics on Nutrient agar	Isolated Organism	Reference
1.	Indole Acetic Acid (IAA), Siderophores, ammonia, exo-polysaccharides, phosphate solubilization	Gram negative	Bluish	*Enterobacter asburae*	Ahmed and Khan (2010)
2.	ACC deaminase, IAA, siderophore, phosphate solubilization	Gram negative	White to cream	*Enterobacter mori*	Kumar et al. (2008)
3.	phosphate solubilization, IAA, exo-polysaccharides,ammonia siderophores, HCN	Gram negative	Cream, yellow to greenish	*Pseudomonas areoginosa*	Ahemad and Khan (2012)
4.	Antifungal activity, siderophore, HCN, phosphate solubilization	Gram negative	Round transparent, smooth colonies	*Pseudomonas putida*	Pandey et al. (2006)
5	Able to form symbiosis with chickpea produce penalate-type sidosphere in response to iron deficiency Nutrient component of medium.	Gram negative	Pink to red Rod shape	*Mesorhizobium ceceri*	Behrrao et al (1997)

RESULTS AND DISCUSSION

Plant Height (cm)

The results showed that when PGPR treatments were significantly greater than the control, the treatments demonstrated a significant response of inoculated seeds. In terms of chickpea plant height, was observed in (T2 *MesoRhizobium ceceri*plus compost) treatment. (34 cm) the highest plant height, while the (T1control) provided the lowest plant height (28 cm) (Figure 3.1).similar finding given by (Janardan. 2010), PGPR are beneficial rhizobacterai for chickpea growth.

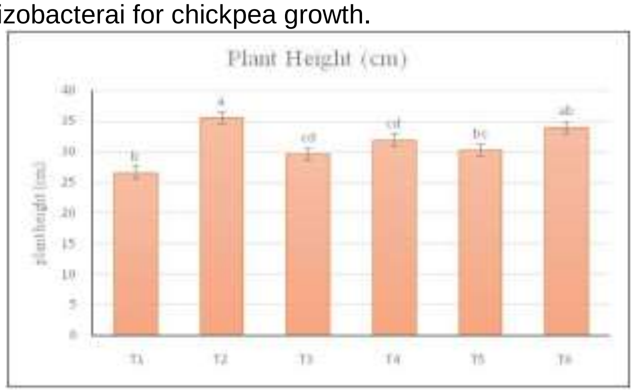

Figure 1:Plant height as affected by PGPR and organic amendments

T_1: control T_2: *Enterobactor mori* T_3: *Enterobacter asburiae* T_4: *Mesorhizobium ceceri*
T_5: *Pesodomonas aeruginosa* T_6: *Pesodomonas putida*

Nodule Count

Data regarding Nodule count showed non-significant response to several rhizobacterial strains inoculation Though, T5 (*Pseudomonas aeruginosa*) revealed that the maximum number of nodules count value was (14.33), while T6 (*Pseudomonas putida*) the lowest value was 11.66. (Figure 3.2). (Katy 2009). Found that the genotype more extensive showed non-significant results of Nodule count.

Figure 2: plant height as affected by PGPR and organic amendments

T_1: control T_2: *Enterobactor mori* T_3: *Enterobacter asburiae* T_4: *Mesorhizobium ceceri*
T_5: *Pesodomonas aeruginosa* T_6: *Pesodomonas putida*

Shoot fresh weight (g)

The shoot fresh weight results showed that the various inoculants improve shoot fresh and dry weight (Figure No 2).The inoculants had a substantial impact on the shoot wet weight (P0.05). T2 (*Mesorhizobium cicero* plus compost)showed the highest wet weight of (39.00 g), which was higher than T3, T4, and T5, while T1 Control had the lowest dry weight (26.66g).similar results (Kanwal et al. 2012), founded that the beneficial rhizobacteria result in improve chickpea seedlings.

Dry weight of Shoot(g)

Statistical data regarding dry weight of shoot showed that organic amendments plus microbe's combination significantly improved dry weight of shoot. The greater shoot dry weight was observed in T2 *Mesorhizobium Cicero* plus compost (17.33g). The least value recorded in T1 control (Figure No.3). Kanwal et al. (2012) investigated that PGPR strains showed a considerable rise in dry weight of shoot (*Cicer arietinum* L) shoot and root.

Figure 3:Shoot dry weight as affected by PGPR and organic amendments.

T_1: control T_2: *Enterobactor mori* T_3: *Enterobacter asburiae* T_4: *Mesorhizobium ceceri*
T_5: *Pesodomonas aeruginosa* T_6: *Pesodomonas putida*

Root Fresh weight (g)

The statistical data showed PGPR plus organic amendments played a significant response on root fresh weight. The highest value was found inT2*Mesorhizobium Cicero* plus compost which was (12.66g).while the lowest value was recorded in T1 Treatment, which was 8.00 (Figure No.4).According to Yadav et al. (2010), rhizobia inoculated with (*Cicer arietinum*) increase root dry weight under different environmental conditions.

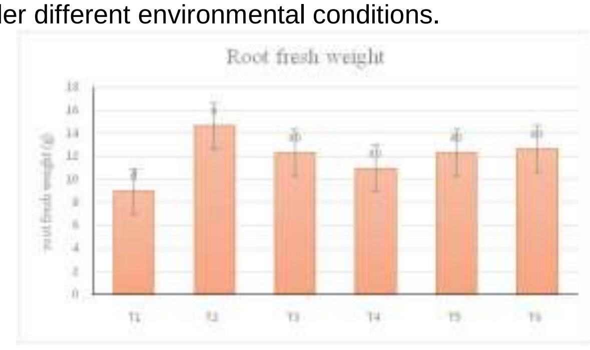

Figure 4: Shoot dry weight as affected by PGPR and organic amendments.

T_1: control T_2: *Enterobactor mori* T_3: *Enterobacter asburiae* T_4: *Mesorhizobium ceceri*
T_5: *Pesodomonas aeruginosa* T_6: *Pesodomonas putida*

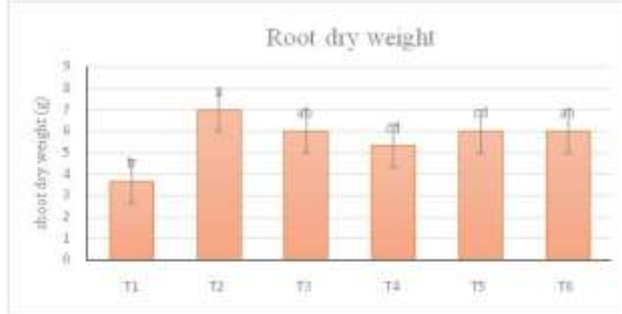

Figure 5:Root dry weight as affected by PGPR and organic amendments.

T_1: control T_2: *Enterobactor mori* T_3: *Enterobacter asburiae* T_4: *Mesorhizobium ceceri*
T_5: *Pesodomonas aeruginosa* T_6: *Pesodomonas putida*

Root dry weight (g)

Statistical analysis of the data regarding dry weight of shoot showed that organic amendments plus microbe's combination significantly improved root dry weight. The Treatment (T2 *Mesorhizobium cicero*plus compost*)(*6.00g) had the highest root weight was found in T1 when compared to control (Figure No. 6). Fatima et al. (2008) reported that a significant rise in root dry weight of chickpea was reported in PGPR inoculation treatments.

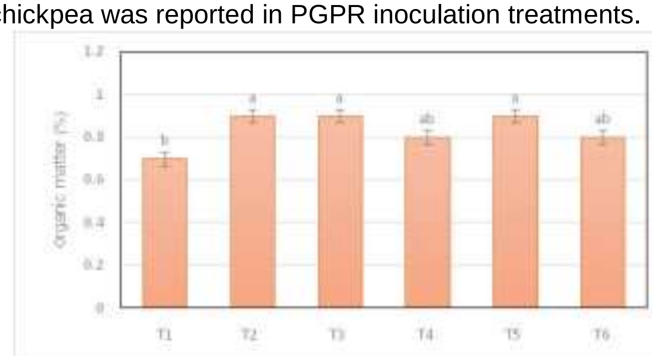

Figure 6: Organic matter (%) as affected by PGPR and organic amendments

T_1: control T_2: *Enterobactor mori* T_3: *Enterobacter asburiae* T_4: *Mesorhizobium ceceri*
T_5: *Pesodomonas aeruginosa* T_6: *Pesodomonas putida*

Post-harvest Soil phosphorus, Nitrogen and Organic matter

Soil phosphorus content absorption increased after inoculation treatment (Figure.7). The highest soil Phosphorus content was found in (*Mesorhizobium Cicero*plus compost) treated soil, followed by (*Enterobacter mori*plus Lentil straw) treated soil, and the lowest value was found in T1 (control). Various researchers (Orhan et al.) reported that rhizobacterial strains have a significant impact on soil phosphorus availability it showed that inoculating Rhizobacterial strains increased phosphate solubilization, which resulted in greater soil phosphorus availability.The results obtained after the crop was harvested had a significant impact on the nitrogen content of the soil.

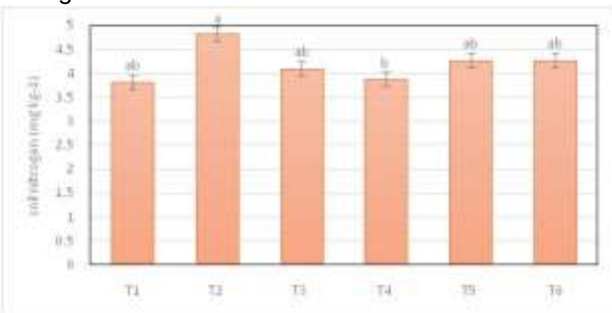

Figure 7:Soil Nitrogen as affected by PGPR and organic amendments

T_1: control T_2: *Enterobactor mori* T_3: *Enterobacter asburiae* T_4: *Mesorhizobium ceceri*

T_5: *Pesodomonas aeruginosa* T_6: *Pesodomonas putida*

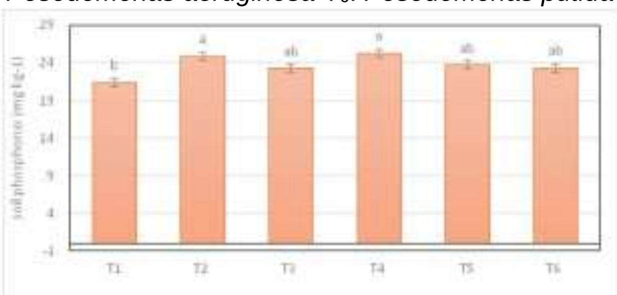

Figure 8: Phosphorus content of soil as affected by PGPR and organic amendments

T_1: control T_2: *Enterobactor mori* T_3: *Enterobacter asburiae* T_4: *Mesorhizobium ceceri*

T_5: *Pesodomonas aeruginosa* T_6: *Pesodomonas putida*

The highest value for soil nitrogen was found in the (*Mesorhizobium ceceri*plus compost) treatment, while the lowest was found in the untreated soil. (Figure No.8.) Ma et al. (2011) reported a similar finding, stating that rhizobacteria significantly increase soil nitrogen. According to the data, inoculants had a significant effect on soil organic matter content (0.05) The highest Organic matter observed in in *Mesorhizobium cecri,*(1.2) which was followed by*Enterobacter asburiae*, and *Pseusomonas aeruginosa* (Figure No.6).It was statistically similar to (Shabaz 2014).reported that the use of organic amendments plus rhizobacterial strains increased soil organic matter content while also improving plant nutrient uptake.

CONCLUSION

The current study found that the screening of different bacterial strains showed significant response while the most promising results were obtained from (*MesoRhizobium ceceri plus compost)*(*Enterobactor mori* plus Lentil straw) treatments plants showed a significant response on Chickpea growth, plant height, shoot dry weight, and root dry weight, when compared to control plants. The use of PGPR Rhizobacterial strains significantly increases the percentage of chickpea plant growth in arid conditions.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors have equal contribution in performing this research.

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