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Original Article

Response of Rhizobacterial strains and organic amendments on chickpea growth

Resposta de cepas de rizobactérias e alterações orgânicas no crescimento do grão-de-bico

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Abstract

Plant Growth Promoting Rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and promote plant growth through a variety of mechanisms such as phosphate solubilization, phytohormones production, antifungal activity and also improve plant growth and yield. Field experiment was carried out to investigate the residual effect of organic amendments plus soil microbes along with integrated nutrient management. (PGPR) are important soil organism that promotes plant growth and yield root colonization is an example of a direct and indirect mechanism. The treatments included control, (inorganic fertilizer and no organic fertilization).Five bacterial strains were identified morphologically and biochemically screened from the rhizospheres of chickpea, lentil, barseem, mungbean, and sesame. The experiment was conducted at the Arid Zone Research Center in D.I.Khan (Pakistan). The majority of isolates resulted in significant increase in shoot length, root length, and dry matter production of *Cicer arietinum* seedlings' shoot and root. The experiment represented that isolates treated plots with rhizobium strain inoculation resulted in greater plant height (35.000 cm) and nodule count (38.00) No of pods per plant^{-1} (44.66) when compared to the control treatment, While (*Mesorhizobium cicero*) along with organic amendments showed significant response the greater root length (50 cm) was observed in T4 treatment. The Performance of rhizobial strains on chickpea germination in an arid environment was found to significantly increase crop germination percentage. This combination thus increases nitrogen and phosphorus uptake in inoculation treated plots. The study found that plots with inoculation treatments yielded significantly higher than non-treated plots Treatment with *Mesorhizobium Cicero* and compost resulted in a higher grain yield (8%) as compared to the control. The greater grain yield was observed in Treatment T4 (183.67).The result showed that use of PGPR have the potential to increase nutrient absorption from soil while improved growth of chickpea.

Keywords: organic amendments, rhizobacteria, chickpea growth and yield.

Resumo

As rizobactérias promotoras de crescimento de plantas (PGPR) são bactérias benéficas que colonizam as raízes das plantas e promovem o crescimento das plantas através de uma variedade de mecanismos, como solubilização de fosfato, produção de fito-hormônios, atividade antifúngica e também melhoram o crescimento e o rendimento das plantas. O experimento de campo foi realizado para investigar o efeito residual de corretivos orgânicos mais micróbios do solo, juntamente com o manejo integrado de nutrientes. PGPR são importantes organismos do solo que promovem o crescimento das plantas e produzem a colonização de raízes, que é um exemplo de mecanismo direto e indireto. Os tratamentos incluíram controle (fertilizante inorgânico e sem adubação orgânica). Cinco cepas bacterianas foram identificadas morfologicamente e bioquimicamente selecionadas das rizosferas de grão-de-bico, lentilha, barseem, feijão-mungo e gergelim. O experimento foi conduzido no Arid Zone Research Center em D.I.Khan (Paquistão). A maioria dos isolados resultou em aumento significativo no comprimento da parte aérea, comprimento da raiz e produção de matéria seca da parte aérea e raiz das plântulas de *Cicer arietinum*. O experimento demonstrou que isolados de parcelas tratadas com inoculação de cepa de rizóbio resultaram em maior altura de planta (35.000 cm) e contagem de nódulos (38,00) n.º de vagens por planta-1 (44,66), quando comparado ao tratamento controle, enquanto (*Mesorhizobium cicero*), juntamente com as alterações orgânicas, apresentaram resposta significativa quanto maior o comprimento da raiz (50 cm) observado no Tratamento T4. O desempenho de linhagens de rizóbios na germinação do grão-de-bico em ambiente árido aumentou significativamente a porcentagem de germinação da cultura. Esta combinação aumenta assim a absorção de nitrogênio e fósforo nas parcelas tratadas com inoculação. O estudo constatou que as parcelas com tratamentos de inoculação produziram significativamente mais do que as parcelas não tratadas. O tratamento com *Mesorhizobium cicero* e composto

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resultou em maior produtividade de grãos (8%) em comparação com o controle. A maior produtividade de grãos foi observada no Tratamento T4 (183,67). O resultado mostrou que o uso de PGPR tem potencial para aumentar a absorção de nutrientes do solo enquanto melhora o crescimento do grão-de-bico.

Palavras-chave: corretivos orgânicos, rizobactérias, crescimento e produtividade do grão-de-bico.

1. Introduction

Pulses are grown on an area of 1.492 million hectares in Pakistan, with a total production of 983,000 tones. Chickpea (Gram) is an important winter pulse crop in Pakistan, and in 2011-12, it was cultivated on 1055 thousand hectares with a production of 291 thousand tones, indicating a 41.3 percent decrease in production compared to the previous crop due to unfavorable weather conditions. Legume crops, by providing a variety of services at the food and production system levels, play an important role in food security and the long-term viability of agro-ecosystems (Stagnari et al., 2017). For the years 2010-11 and 2010, chickpea production was 5 M+t compared to 5.6 M+t the previous year representing a 6.9 percent decrease for the period 2010-11, owing primarily to unfavorable weather conditions (Hirdyani, 2014). Legumes are important because of their nitrogen-fixing properties. Pulses in cropping systems can boost symbiotic N fixation and improve soil phosphorus levels.. Atmospheric nitrogen, fixation N2 in legume crops by Rhizobium leguminosarum, accounts for half of the world's annual 175 million tons of gross organic nitrogen fixation. Particularly in legumes. Nitrogen in the atmosphere, N2 fixation in legume crops by Rhizobium leguminosarum, accounts for half of the world's annual 175 million tons of gross organic nitrogen fixation. Depending on the cultivar, bacterial strain, and natural components, the approximate amount of nitrogen fixed in legumes contributes up to 176 kg N ha per year. (2014) (Gopalakrishnan et al, 2015).

Phosphorus fertilization availability by bacterial inoculation research is ongoing worldwide, with a greater emphasis on discovering new characteristics of these bacteria (Ma et al., 2011; Wani and Khan, 2010). The role of soil phosphate fertilization is critical. A significant amount of agricultural land is underutilized due to phosphate (P) deficiency in the soil.

Up to 80 percent coefficients are attributed to mycorrhizal fungi and nitrogen-fixing bacteria alone. Annually, plants acquire 7% of all nitrogen and 75% of all phosphorus (van der Heijden et al,2008)

The use of agricultural waste such as substrate is an ancient essential for either adding profit to this type of waste after depressing the price of seedling production. Organic fertilizers improve the soil's chemical, biological, and physical structure, as well as its organic matter. The presence of high Organic matter improves soil structure, water and nutrient absorption, reduces erosion, and encourages plant growth as well as organic fertilizer increases soil organic carbon immediately, which is generally proportional to the amount of carbon applied (Chantigny et al., 1999). Rhizobacteria that promote plant growth are important microorganisms found in the rhizosphere, on root surfaces, and in relationships with roots that have the potential to significantly improve the nature or extent of plant growth. (*Arthrobacter,Alcaligenes*, *Burkholderia, Pseudomonas, Azospirillum, Azotobacter), Klebsiella, Enterobacter Bacillus*, and Serratia have all been identified as PGPR to improve plant growth (Kloepper et al., 1989).

Rhizobacteria that promote plant growth are microorganisms that not only promote plant growth but also have a significant impact on soil. However, in addition to increased crop yields in the record of controlling various pollutants and pathogens (Ahemad and Kibret, 2014). Fertility and nutrient availability in soil (Glick, 2012). PGPR, for the most part, play a role in plant growth by directing plant hormone levels and bio controlling various plant pathogens. Scientists over the world use rhizobacteria to boost crop yield. Furthermore, extensive research is being conducted all over the world in order to discover new characteristics of these bacteria. The (N2) is ranked in two-thirds (Ma et al., 2011; Wani and Khan, 2010).

2. Materials and Methods

Field study was conducted at (AZRC) D.I. Khan, to observe the effect of PGPR Strains growth and yield parameters.

2.1. Rhizobacterial strains preparation

The rhizobacterial strains were obtained from legume fields. Roots were uprooted from lentil and chickpea fields' rhizospheres Plant samples were transported to the laboratory for further analysis. They were brought up on the (GPA) media. All strains were transferred to (Yield Menitol Agar) (YMA) after Inoculation.

2.2. Broth culture media preparation

On agar plates, free-living bacterial strains were cultured and strains with the highest exo-polysaccharide production were chosen based on visual inspection. These bacteria were grown on agar medium before being transferred to broth media. These chemically sterilized pink and large-sized nodules were punctured with a sterilized needle and streaked for 72 hours on yeast extract mannitol agar (YMA) media plates, which were incubated at 28 °C. After 72 hours, the growth on the Petri dish was visually observed, and well-grown colonies were found.

Following the completion of the bacterial identification process.

Phylogenetic identification of five rhizobacterial strains was done from Micro Gen® Korea Broth media were used to treat the seeds. Chickpea socked seed in this media was grown in the field. Experiment was laid in (RCBD) two factorial Randomized Complete Block Design with three replicates. Chickpea variety named: Nifa 2005 was sown. Each plot was applied N and P @:20:50 kg/ha^{-1}, respectively. Along with organic fertilization

(Lentil straw, compost, mungbean and wheat straw) was applied to all the respective plots except control.

2.3. Treatment detail

Organic amendments to the main plots were as follows:
S_1: Control
S_2: Mungbean straw
S_3: Compost
S_4: Lentil straw
S_5: wheat Straw
S6:Mungbean straw
The following rhizobacterial strains used:
T_1: Control
T_2: *Enterobacter asburiae*
T_3: *Enterobacter mori*
T_4: *Mesorhizobium ceceri*
T_5: *Pseudomonas aeruginosa*
T_6: *Pseudomonas putida*

2.4. Statistical analysis

Data collected during the study was statistically analyzed using the Statistix 9.1 software. Procedure used by (Steel et al. 1997). For calculating analysis of variance and least significant difference for mean comparisons was followed

3. Result and Discussion

3.1. Plant height (cm)

Statistical data regarding plant height showed significant differences for plots treated with organic amendments as compared with control. Treatment T4 (*Rhizobium ceceri* + Mungbean straw) produced the greater plant height which was (35.000 cm), representing in (5.67%) increase over the control. Treatment T1 control showed lowest plant height. (24.33).

The interaction of organic amendments and inoculation with chemical fertilizer produced significant results.

The results are similar to (Zahran, 2001) reported that the inoculation of beneficial microbes response with plants greatly improved the plant growth as compared to inoculated treatments.

3.2. Nodule count

Organic amendments, in combination with rhizobacterial strains, demonstrated significant results. Response on nodule counting. . Nodules count showed significant results as compared to the control, Inoculation with organic amendment resulted in the greatest increase in Nodule counting, the highest value (38.00) was found in Treatment T4 while Control T1 showed the lowest value (21.66). Ahmad et al. (2014) reported that this type of association between legume and *Rhizobium* has been well documented. and it has also been confirmed that it plays a significant role in nodule formation in various legumes.

3.3. No of pods per plant^{-1}

Data regarding number of pods per Plant^{-1} was significantly increase in the presence of inoculation and organic amendments. When combined with organic amendment, inoculation resulted in a greater increase in pods per plant than the control (Table 2 . The Treatment T4 (*Rhizobium ceceri* in combination with Wheat straw) had the highest value (44.66) and the lowest value (23.33) was found in Treatment T1 (Control +mungbean straw) (Fatima et al., 2008). Founded that PGPR inoculating chickpea pods with rhizobia increased plant growth, dry matter, and pod number.

3.4. Root length (cm)

Root length was significantly increased (Table 2). Organic amendment and inoculation had a significant impact on root length. T6 (*Pseudomonas putida* plus Compost) had the highest value (46.66), while T1 (Control+Mungbean straw) had the lowest (25.00) These findings are supported by Ahmad et al. (2011) who found that PGPR and rhizobia co-inoculation increased root length and improved water uptake from root zone.

3.5. Plant fresh and dry weight (g)

The data regarding plant fresh and dry weight was non-significant (Table 1). Treatment T2 shows the highest value

Table 1. 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	6.00 ab	7.66 ab	8.33ab	7.66 ab	17.66 e	30.66 bc	25.33 cd	26.33 cd
T2	8.33 ab	8.33 ab	7.33 ab	7.66 ab	22.66 de	23.66 de	34.33 ab	40.33 a
T3	7.00 ab	7.66 ab	7.66 ab	8.66 a	21.33 de	25.00 cd	23.66 de	21.33 de
T4	7.00 ab	7.33 ab	7.33 ab	9.00 a	37.66 ab	22.00 de	25.33 cd	22.66 de
T5	7.00 ab	7.33 ab	7.33 ab	9.00 a	22.66 de	21.66 de	19.66 e	25.66 cd
T6	7.00 ab	7.33 ab	7.66 ab	8.66 a	20.66 e	23.66 de	21.00 e	22.33 de
LSD	2.1693				9.6057			

LSD - (Least significant Difference). g – (Gram).

Table 2. Effect of crop residue along with PGPR inoculation on chickpea plant height, no of pods per plant, 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	23.33 d	30.33 ab	23.33 d	28.66 ab	23.33gh	30.66 cd	31.33 cd	34.00 bc	25.33 g	33.33 de	32.33 ef	29.33 fg	21.66 ij	23.00 hj	20.00 j	27.33 ei	129.00 de	140.67 de	158.67 ab	143.00 ab
T2	27.00 bc	32.00 ab	31.33 ab	31.00 ab	41.33ab	34.33 bc	36.33 ab	23.00 h	43.00 ab	39.00 bc	37.66 cd	47.66 ab	31.66 cg	25.33 gj	27.00 fi	35.33 abc	132.00 cd	179.00 ab	132.00 cd	127.00 de
T3	30.33 ab	32.00 ab	30.33 ab	31.33 ab	33.00 bc	34.33 bc	38.33 ab	42.00 ab	37.33 cd	38.66 bc	41.66 ab	44.00 ab	33.66 bc	23.33 hi	32.00 cf	34.00 ad	167.00 ab	164.33 ab	128.00 de	111.67 e
T4	34.33 ab	34.66 ab	35.00 a	35.00 a	32.66 bc	24.33 fg	27.00 de	44.66 a	46.66 ab	40.00 bc	43.33 ab	41.00 bc	35.00 ad	26.66 fi	31.00 cg	38.66 ab	134.33 bc	179.67 a	127.00 de	153.33 ab
T5	28.33 ab	32.66 ab	31.33 ab	32.66 ab	29.66 cd	25.33 ef	26.33 ef	40.33 ab	47.66 ab	42.00 ab	44.66 abc	43.66 ab	36.00 ac	28.66 dh	32.33 bf	36.00 ac	131.33 cd	141.67 ae	138.00 ab	176.33 ab
T6	30.66 ab	31.66 ab	31.333 ab	31.66 ab	31.33 cd	24.33 fgh	27.33	32.33 bc	49.66 ab	43.33 ab	46.33 ab	52.66 a	32.66 bf	25.33 gj	28.66 dh	35.66 ac	129.60 de	148.00 ab	136.33 ab	141.67 ab

Cm (Centimeter). mg/kg (Milligram per Kilogram).

(25.33). (*Enterobacter asburiae* + Wheat straw), followed by T4, while the lowest value was found in T3 (Control along with Compost) (20.66) Inoculation, in combination with organic amendment, resulted in a non-significant in Plant dry greater value was found in T1 (9.00) which was at par with T1 control. While the lowest value was observed in T5 Treatment.). similar finding Karnwal and Kumar (2012) reported that most of isolates resulted in a significant increasing of dry matter weight of shoot and root of Chickpea seedlings.

3.6. Grain yield kg/plot

Statistical data of grain yield revealed that it is positively increased by organic amendment in addition to inoculation throughout the experiment. Grain yield was significantly increased by 8%. The greater value was observed in Treatment. T4 (*Rhizobium ceceri* Plus Wheat straw) (183.67) and followed by T2 (*Enterobacter asburiae* along with Mungbean straw), T3 (*Enterobacter mori*), T6 (*Pseudomonas putida*), while the lowest value was observed in T6 Treatment. Ahmad et al. (2014) reported that this type of relationship between legumes and rhizobium is well known and documented. And it has also been confirmed that it plays a significant role in yield and nodule formation in various legumes.

3.7. Soil available N P and (organic matter %)

During the experiment, bio fertilizers plus organic amendment treatments resulted in a significant increase in soil available nitrogen, phosphorus, and organic matter percentage (Figure 1). Phosphorus content in soil increased significantly in seed treated with inoculation treatments compared to controls. The highest phosphorus content in soil was found in treatment 4, while the lowest was found in T1Control. The outcomes are consistent with Verma et al. (2010) PGPR has been shown to solubilize precipitated phosphates and increase phosphate availability to chickpea, which represents a possible mechanism of plant growth promotion in the field (Figure 2). The inoculants also have a significant impact on the soil's nitrogen content. Similarly, the highest value was observed in the T4 treatment, and the least value was recorded in the T1 Control According to Ma et al. (2011).

Rhizobacteria increase to some extent, the nitrogen content of the soil. Gamalero et al. (2004) reported PGPR inoculation improves Nitrogen percentage (Figure 3).

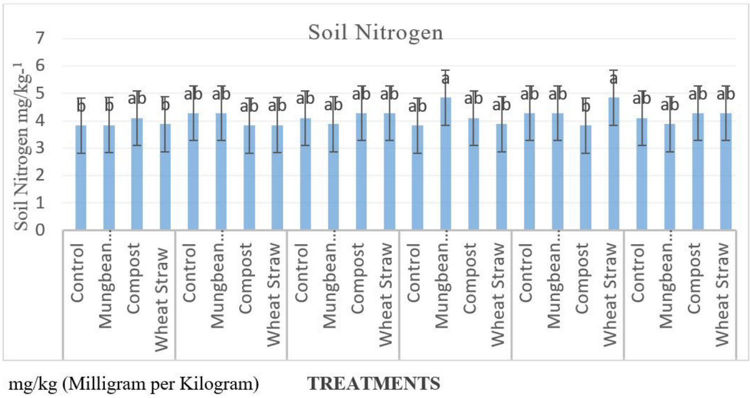

Figure 1. Soil Nitrogen as affected by Rhizobacterial strains. T_1: control. T_2: Enterobactor asburiae. T_3: Enterobacter mori. T_4: rhizobiu ceceri. T_5: Pesodomonas aeruginosa. T_6: Pesodomonas putida.

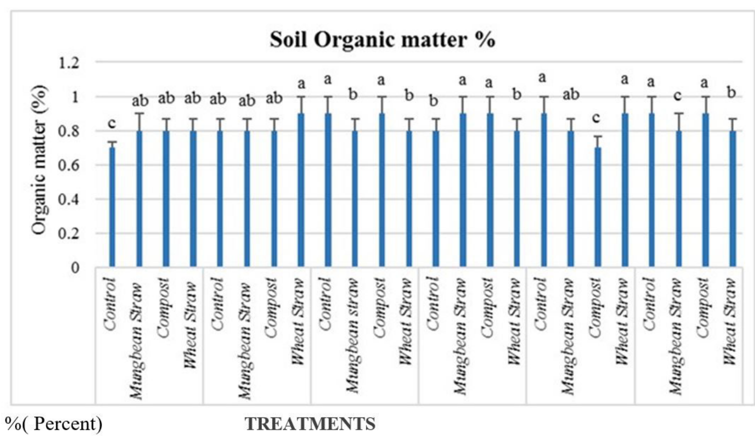

Figure 2. Soil organic matter as affected by Rhizobacterial strains. T_1: control. T_2: Enterobactor asburiae. T_3: Enterobacter mori. T_4: rhizobiu ceceri. T_5: Pesodomonas aeruginosa. T6: Pesodomonas putida.

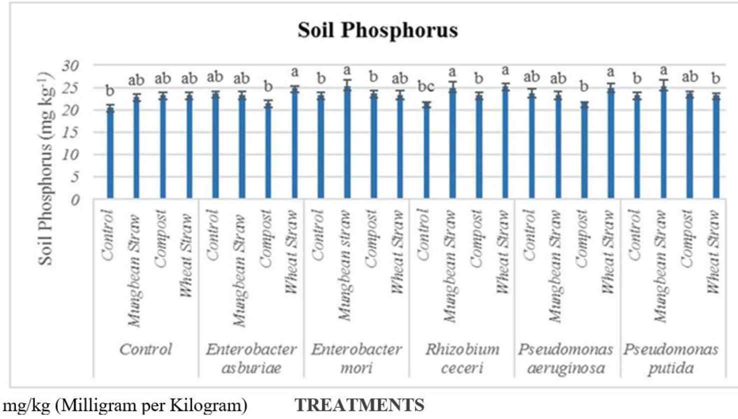

Figure 3. Soil phosphorus as affected by Rhizobacterial strains. T_1: control. T_2: Enterobactor asburiae. T_3: Enterobacter mori. T_4: rhizobiu ceceri. T_5: Pesodomonas aeruginosa. T_6: Pesodomonas putida.

The data show that the inoculant responded positively to increasing soil organic matter because the values changed significantly, with the greatest observed in treatment T4 and the lowest recorded in treatment control.

The findings are consistent with Shahbaz et al. (2014). It was suggested that the use of bio fertilizer increased organic matter and essential plant nutrients in the soil. Because of the phosphate solubilization, PGPR can also enhance and improve the availability of soil phosphorus. Crop residue and PGPR Inoculation improved soil potassium levels, according to the findings. Data show that inoculants also play an important role in soil organic matter because the values change significantly, with the greatest observed in treatment T1 and the lowest recorded in treatment control. The outcomes are consistent with Shahbaz et al. (2014). Bio fertilizer application to the soil increases organic matter and essential plant nutrients in the soil.

4. Conclusion

It can be concluded that PGPR (*Mesorhizobium ciceri* + compost) strains were the most effective in improving plant growth parameters, whereas rhizobacterial strains showed a positive response on soil properties. Other isolated strains and inoculants have revealed that the shoot and root parameters of chickpea have been optimized. The use of Rhizobacterial strains in conjunction with (wheat straw, mungbean straw, lentil straw, and compost) demonstrated the most effective role in soil nutrient uptake by plants. As a result, various inoculants were used, with *Mesorhizobium ciceri* and compost being the most effective. *Mesorhizobium ciceri* may be recommended for increasing soil nutrients while also optimizing the agronomic parameters of the Chickpea crop.

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