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Co-inoculation of *Bradyrhizobium* and Phosphate Solubilizing Microbes on Growth Evaluation of Groundnut under Rain-fed Conditions

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Abstract

Plant growth-promoting bacteria (PGPB) can improve plant development and protect plants from diseases and abiotic stresses. Plant-bacterial interactions in the rhizosphere are important factors in soil fertility and plant health. Symbiotic nitrogen-fixing bacteria include the cyanobacteria of the genera *Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Allorhizobium*, *Sinorhizobium* and *Mesorhizobium*. Therefore, to investigate the effect of co-inoculation of *Bradyrhizobium* and phosphate solubilizing microbes (PSM) on groundnut crop under field conditions using normal soil, divided into eight different study groups i.e., control (T₁), *Bradyrhizobium* isolate -1 (T₂), *Bradyrhizobium* isolate -2 (T₃), *Bradyrhizobium* isolate -3 (T₄), Phosphate solubilizing microbe (PSM) (T₅), T₂ + PSM (T₆), T₃ + PSM (T₇), T₄ + PSM (T₈). The results showed maximum groundnut pod yield (2428 kg ha⁻¹) was obtained in treatment where inoculation with bacterial isolate-2 applied as compared to control. In case of plant height and shoot dry biomass, maximum response was observed in T₇ (59.4 cm, 4733 kg ha⁻¹) as compared to control i.e., 40.5 cm, 3156.7 kg ha⁻¹ respectively. It was concluded that this technique might be useful and applicable to cut down the high input cost of phosphate fertilizers for the production of other crops also.

Keywords: Groundnut, biochemical process, P-fixation, root-microbe interaction, soil bacteriology

Introduction

The expectations placed on agriculture to produce future food will be one of the major problems confronting the agricultural community as the world's human population continues to grow (Ahmadzai *et al.* 2021). To tackle this issue, a significant amount of research concentrating on the soil biological system and the agroecosystem as a whole is required to better understand the complex processes and interactions that regulate agricultural land stability (Liu *et al.* 2022). The green revolution has been one of humanity's most profoundly successful human endeavours that resulted in the security of global food security and transformed certain poor countries (Boyacı-Gunduz *et al.*, 2021). However, the world's food security has once again been endangered by the constant and worrisome growth in human population. However, a second green revolution is urgently needed to raise food production by roughly 50% in the next 20 years in order to keep up with population growth (Aliyu *et al.*, 2021). The rhizosphere is the soil area where microorganism-mediated activities are affected especially by the root system (Fazeli-Nasab

et al., 2022). Organic chemicals released by roots function as signalling molecules, attracting helpful microorganisms and repelling harmful microbes. Peanut (*Arachis hypogaea* L.) or groundnut cultivation on the same land adds to the build-up of root exudates, which leads to an increase in soil pathogens and a reduction in production. These crops play an important role in food security and the long-term viability of agro-ecosystems by offering a variety of services at the food and production system levels. Traditionally, organic and inorganic fertilizers have been used to correct nutrient deficiencies and maintain nutrient balance in farm fields (Tripathi *et al.*, 2022). Rhizobia bacteria (genera *Bradyrhizobium*) because of their symbiotic relationships with legumes, they are responsible for the majority of the nitrogen fixed in the atmosphere on the planet (Piromyou *et al.* 2021). These bacteria are only species that can invade intercellular spaces of groundnut without any infectious harm to plant (Shrama *et al.*, 2022). Co-inoculation is the combined application of PGPRs and other bacteria, bestowed with some specialized functions, to increase the nodulation rate, plant

growth, and plant tolerance to adverse environmental conditions (Silambarasan *et al.*, 2022). Phosphorus (P) is a major and essential macronutrient for food production and it plays a key role in different growth processes occurring in plants, such as root production, flowering, seed formation, photosynthesis, and maturation. The unavailability of soil P to plants due to binding to soil mineral particles and elements (e.g., calcium (Ca), magnesium (Mg), aluminium (Al), iron (Fe)) present in the soil causes severe crop yield losses (Lun *et al.*, 2018; Roy *et al.*, 2016). Total phosphorus in our soil is more than 1500 mg kg⁻¹, while the available-P is only 5 to 10 mg kg⁻¹ due to fixation. It is a big problem for the researchers to make sure the availability of P in soil from Total-P rather than fertilizers. Therefore, the current study aimed to investigate the efficiency of *Bradyrhizobium* and phosphate solubilizing microbes (PSM) on growth and yield of groundnut crop under rain-fed conditions. Furthermore, the Characterization of *Bradyrhizobium* and phosphate solubilizing bacteria were done in lab before their application as seed coating.

Material and Methods

Retrieval of Rhizobia: In order to evaluate the effect of *Bradyrhizobium* and PSM, groundnut rhizosphere samples were collected from different locations and *Bradyrhizobium* spp. were isolated in laboratory. Three efficient *Bradyrhizobium* isolates were selected for checking their effect on groundnut crop separately as well as in combination with PSM.

Bacterial strains and culture growth conditions: Field trial were conducted with three repeats in RCBD. Recommended dose of NPK (25:80:25) were applied as basal dose. Completely randomized block design was used. Groundnut crop was sown during

Kharif 2019 **Inoculation Experiments:** The whole study field trial was conducted at the Soil and Water Conservation Research Institute, Chakwal, using normal soil. The study applied eight (08) different sets of treatment represented by T i.e., Control Treatments (T₁), *Bradyrhizobium* isolate -1 (T₂), *Bradyrhizobium* isolate -2 (T₃), *Bradyrhizobium* isolate -3 (T₄), Phosphate solubilizing microbe (PSM) (T₅), T₂ + PSM (T₆), T₃ + PSM (T₇), and T₄ + PSM (T₈) respectively. Additionally, *Bradyrhizobium* and PSM were characterized in the lab before being used as seed coatings.

Plant Characteristics: Different parameters related to plant were measured like plant height, Fresh weight, no. of pods, pods fresh weight, pods dry weight.

Statistical analysis: With three repetitions, the experiment was run in a RCBD. Analysis of variance (ANOVA) (Steel *et al.*, 1997) was applied to statistical analysis to obtained data followed by using of Statistix 8.1 software. Moreover, at 5% probability levels, the Least Significance Difference (LSD) values between the treatments were computed.

Results

Yield Study: Data revealed that maximum groundnut pod yield (2428 kg ha⁻¹) was obtained in treatment where PSM inoculation was done with bacterial Isolate-2 (T₇) as compared to control. Whereas, the minimum increase in yield was observed on bacterial isolate-3 Inoculation (T₄) i.e., 1444 kg/ha, however it was statistically at par to control (1234.6 kg/ha) as shown in Figure 1. The overall study represents an increase in yield of groundnut as T₇ > T₈ > T₃ > T₂ > T₆ > T₅ > T₄ > T₁ > LSD

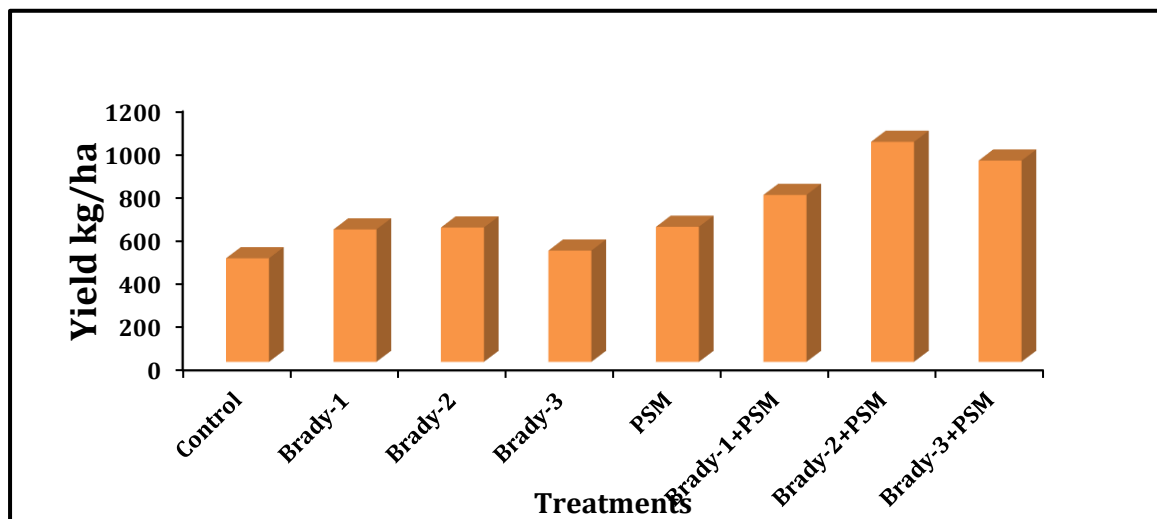


Fig. 1: Grain yield of groundnut in kg/ha investigated at eight different treatments

Control Treatments (T₁), *Bradyrhizobium* isolate -1 (T₂), *Bradyrhizobium* isolate -2 (T₃), *Bradyrhizobium* isolate -3 (T₄), Phosphate solubilizing microbe (PSM) (T₅), T₂ + PSM (T₆), T₃ + PSM (T₇), and T₄ + PSM (T₈).

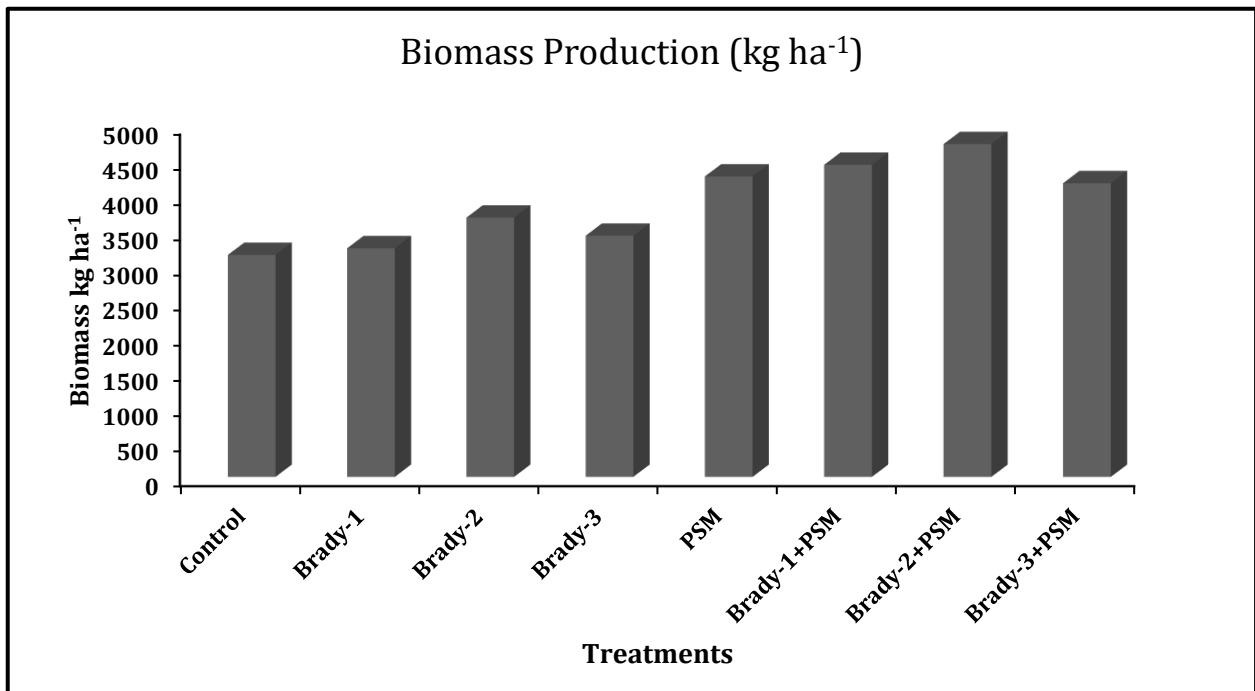


Fig. 2: Biomass yield of groundnut in kg/ha investigated at eight different treatments i.e., Control Treatments (T₁), *Bradyrhizobium* isolate -1 (T₂), *Bradyrhizobium* isolate -2 (T₃), *Bradyrhizobium* isolate -3 (T₄), Phosphate solubilizing microbe (PSM) (T₅), T₂ + PSM (T₆), T₃ + PSM (T₇), and T₄ + PSM (T₈).

Biomass evaluation: The number of biomasses generated through organic (plant) is of significant importance as it is used to generate energy. In current study a wide increase in the production of biomass was observed. The maximum response was observed in T₇ treatment comprising of Brady-2+ PSM (4733 kg ha⁻¹). While the minimum was observed in T₂ (3251.3 kg ha⁻¹) still high then control treatment i.e., 3156.7 kg ha⁻¹ respectively. The increase in biomass throughout the treatment was as T₇ > T₆ > T₅ > T₈ > T₃ > T₄ > T₂ > T₁ > LSD as shown in figure 2.

Plant Pods Analysis: The pod production in plant was also analysed in current study. The results revealed that increase in the production of number of peanut pods (31.4) was observed in T₇. While the least no (21.8) in case of applied inoculums was observed in T₄. As far as control treatment was concerned, it gave minimum no of pods ((15.0). The detailed results are shown in Figure 3. The increase in pods production in current study was in order of T₇ > T₆ > T₈ > T₅ > T₃ > T₂ > T₄ > T₁ > LSD. The soil with brady strain 1 and 2 were statistically at par to each other, revealing that such strains does not have significantly different effect on no of pods. However, brady strain 2 in combination with PSM rendered best

results followed by Brady strain 3 with PSM combination.

Number of Nodules Investigation: Furthermore, the number of nodules in each treated plot was investigated. The statistical analysis revealed an increase in nodules in treatment with T₇ (25.2) whereas, T₅ treatment results in the minimum nodules formation i.e., 12.3 while still have the larger production than control i.e., 8.6 as shown in Figure 4. The increase in nodules production in current study was as T₇ > T₈ > T₆ > T₃ > T₅ > T₄ > T₂ > T₁ > LSD.

Height Evaluation of Plant: The height of peanut plant before and after was also evaluated. The present study revealed that the height of plant was increase after the T₇ i.e., 59.4. However, the minimum effects were observed in pant the after the treatment with T₂ as 46.1, still high then control treatment i.e., 40.0 respectively as shown in Figure 5. The increase in height of plant was overall observed as T₇ > T₃ > T₈ > T₆ > T₅ > T₄ > T₂ > T₁ > LSD.

The overall identified results of current study are summarized in Table 1 highlighting the calculated variation in groundnut after the different eight treatments classes.

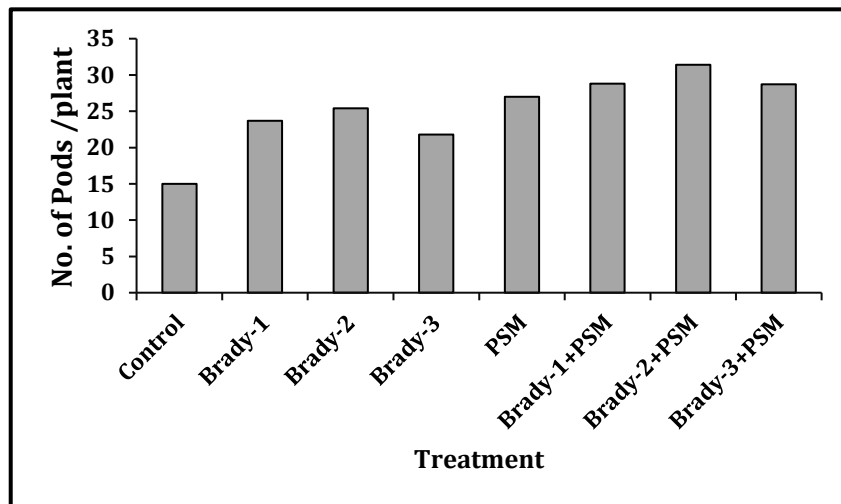


Fig. 3: Pod production of groundnut investigated at eight different treatments i.e. Control Control Treatments (T₁), *Bradyrhizobium* isolate -1 (T₂), *Bradyrhizobium* isolate -2 (T₃), *Bradyrhizobium* isolate -3 (T₄), Phosphate solubilizing microbe (PSM) (T₅), T₂ + PSM (T₆), T₃ + PSM (T₇), and T₄ + PSM (T₈).

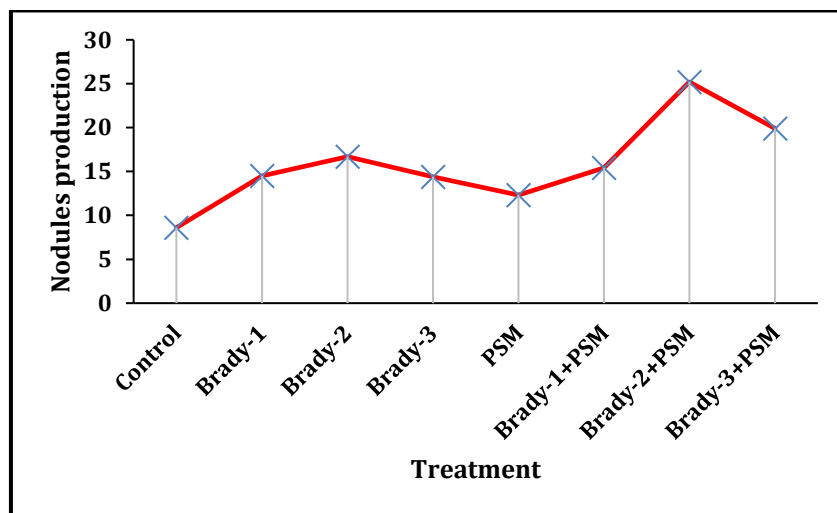


Fig. 4: Nodule's formation in groundnut in kg/ha investigated at eight different treatments Control Treatments (T₁), *Bradyrhizobium* isolate -1 (T₂), *Bradyrhizobium* isolate -2 (T₃), *Bradyrhizobium* isolate -3 (T₄), Phosphate solubilizing microbe (PSM) (T₅), T₂ + PSM (T₆), T₃ + PSM (T₇), and T₄ + PSM (T₈).

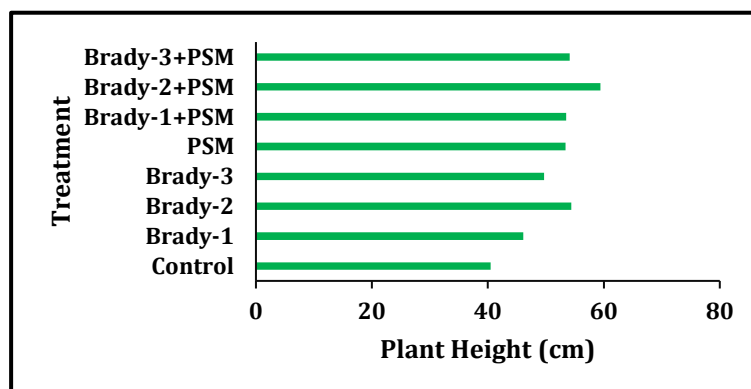


Fig. 5: Height observed in groundnut investigated at eight different treatments. Control Treatments (T₁), *Bradyrhizobium* isolate -1 (T₂), *Bradyrhizobium* isolate -2 (T₃), *Bradyrhizobium* isolate -3 (T₄), Phosphate solubilizing microbe (PSM) (T₅), T₂ + PSM (T₆), T₃ + PSM (T₇), and T₄ + PSM (T₈)

Table 1. Plant characteristics after treatment incorporation.

Treatments	Plant Height (cm)	No. of Pods plant ⁻¹	Grain Yield (kg ha ⁻¹)	Biomass Yield (kg ha ⁻¹)	No. of Nodules
T ₁ : Control	40.5 ab	15.0 e	1234.6 d	3156.7 c	8.6 d
T ₂ : Brady-1	46.1 ab	23.7 de	1687.0 d	3251.3 bc	14.5 cd
T ₃ : Brady-2	54.4 ab	25.4 bcd	1786.0 c	3687.3 bc	16.7 ab
T ₄ : Brady-3	49.7 ab	21.8 cde	1444.0 d	3429.8 bc	14.4 cd
T ₅ : PSM Inoculation	53.4 b	27.0 abc	1585.0 d	4273.1 bc	12.3 cd
T ₆ : Brady-1 +PSM	53.5 ab	28.8 ab	1650.0 bc	4438.3 b	15.4 bc
T ₇ : Brady-2+ PSM	59.4 a	31.4 a	2428.0 a	4733.0 a	25.2 a
T ₈ : Brady-3+ PSM	54.1 ab	28.7 ab	1987.0 b	4176.3 bc	19.9 bc
LSD	5.28	4.76	312.18	690.48	5.1074

Discussion

Bradyrhizobium strains that are photosynthetic have been shown to generate nitrogen-fixing nodules on groundnut stems and roots. The identification of the two partners involved the symbiosis between rhizobia and groundnut i.e., N₂ fixing symbiosis. Rhizobial symbiosis causes the development of nodules in the roots of host plants and fixes nitrogen to supply ammonium-N to the plants (Franche *et al.* 2009; Piromyou *et al.* 2021). Plant-microbe interactions in the rhizosphere are important factors that influence plant health, production, and soil fertility (Stagnari *et al.* 2017). As a result, biological fertilization by the application of particular microorganisms should be promising for both crop yield and economic-environmental sustainability in legume-based cropping systems. In current study we have utilized *Bradyrhizobium* in co-inoculation with PSM to analyse the yield production under rain-fed condition. The significant effect of *Bradyrhizobium* and PSM with other combinations were generated. The current study results showed increment in plant height due to supply of more nutrients while maximum plant height observed in T₇ where a combination *Bradyrhizobium* isolates-2 and PSM were applied. Similar results were reported by Ijaz *et al.*, (2019). However, the mostly minimum number of productions were observed in T₄, and T₂. The applied parameters were also study for Berseem, hence strengthens our identified results (Ijaz *et al.* 2019). *Bradyrhizobium* has been widely used against *Vigna radiata* plant for its hormone's modulation effects, against groundnut nodules productions (Knezevic *et al.*, 2022), for the *Oryza breviligulata* as an endophyte (Chaintreuil *et al.* 2000), and even against non-legumes plant (*Raphanus sativus*) (Antoun *et al.* 1998). Importantly, Alves *et al.*, and Adesemoye *et al.*, also emphasis on the use of inoculants methods to improve the management of plants product that may reduce the use of chemical fertilizers (Alves *et al.* 2003; Adesemoye *et al.* 2009). Danhorn *et al.* (2007), Meneses *et al.* (2011) and Alquéres *et al.* (2013) had study the effects of PGPB effects and concluded its effects to be closely related to that soils microbes. Similar to our results a high interaction had been observed between the added bacterium and plant genotypes in cowpea (Marinho *et al.* 2014; Marinho

et al. 2017; Alcantara *et al.* 2014), and peanut (de Melo

et al. 2016). The variation in results can be observed because of inoculant relation with the type of crop.

Legumes are known to fix nitrogen through biological means. Increase in nodulation as well their action is positively affected through co inoculation of microorganisms is an effective to improve nitrogen availability as well as agricultural productivity in a long run (Bai *et al.* 2002; Abdel-Wahab *et al.* 2008). The similar pattern was observed in our study as PSM not only increased the yield but also added more nodules that will help in soil sustenance. The basic phenomenon identified for effectiveness in legume production is the production of phytohormones which stimulates root growth, ultimately making more room for microbial infection and nodulation (Vessey and Buss, 2002). Rhizo microorganisms produce high amounts of growth hormones like indole acetic acids responsible for root growth and enhancing surface area. the higher production as revealed in our results is attributed to the stimulated root growth due to addition of PGPRs. The same is stated previously and indole secretion is the vital hormone for this effectiveness (Glickmann *et al.* 1998; Zahir and Arshad, 2004; Verma *et al.* 2010).

As far as the inoculation of *bradyrhizobia* strains is concerned, it also impacted the growth positively (Fig. 5) but less as compared to the its addition along with PSM. A great improvement in nodule no and other parameters were observed in treatments with *bradyrhizobia* in comparison to control.

Previous studies also claimed that inoculation with *bradyrhizobia* (identified effective strains) in peanut. However as previously stated by Minamisowa *et al.* (1992) and Vlassak and Vanderleyden (1997) , it is important to inoculate the soil with effective strains as the soils of certain areas comprise of indigenous strains of bacteria that are abundant in number and makes the inoculation less successful.

Improvement of nodulation pattern may occur by providing the peanut-brady rhizobia system with some synergistic substances, such as auxins, flavonoids-like compounds and siderophores, which enhance root proliferation and provide more infection sites occupied by rhizobia and in synchronism enhancing the survival and activity of microsymbiont

in the peanut rhizosphere. A range of evidences have been reported by many investigators on PGPR stimulation of nodulation, as well as creation of more infection sites on the hairs and epidermis of the leguminous plant roots (Vessey and Buss, 2002; Abdel-Wahab et al. 2008; Verma et al. 2010).

Direct and indirect effects of PSM is stated by previous studies influencing the plant during various phases of its life cycle (Gamalero and Glick, 2011; Biswas et al. 2022). The direct affect comprises of nitrogen fixation which ensures the conversion of nitrogen into available forms for the plant (Kafeel et al., 2023), phosphate solubilization (Alori et al. 2017) and the release of phytohormones (Egamberdieva et al. 2017). along with enhanced mineral availability (Korir et al. 2017). Nitrogen fixation occurs by the action of nitrogenase enzyme (oxygen sensitive) responsible for converting atmospheric into biological available forms to the plants or else release into the soil. As our results revealed that *bradyrhizobium* strains was able to produce higher yields in comparison to control, it may be due to the effect of rhizobia in releasing of nitrogen that rendered beneficial results for the overall growth of the plant. Phytohormones like cytokinin's, auxins and gibberellins enhance crop growth. Another property of rhizobia and beneficial microbes is the production of siderophores.

The higher growth parameters were observed in treatment in which PSM were included with *bradyrhizobia*. It may be attributed to the combined ability of *bradyrhizobia* to fix nitrogen and PSM to release the otherwise unviable phosphorus to the plants. Both nutrients are required for efficient growth and higher fruit production which is also seen in current study.

Both inoculations indirectly affect the stomatal regulation, adjust osmotic pressure, modify root morphology under stress environment (Ngumbi and Kloepper, 2016; El-Esawi et al. 2018; Ryu et al. 2004; Sharma et al. 2016). The strong plant is less prone to infectious disease caused by pathogens they also produce antagonistic substances like acetoin, 2-3 butanediol, hydrogen cyanide, phenazine and diacetylphloroglucinol and siderophores. The lytic enzymes viz., chitinase and gluconate produced by these PGPRs can degrade the cell-wall of fungal pathogens, thus inducing systematic resistance throughout the entire plant system.

Conclusion

The current study comprises of filed study performed at Chakwal for the analysis of yield production of groundnut. The study was aimed to evaluate the effect of *Bradyrhizobium* in co-inoculation with PSM. the study was conducted to improve the yield production in groundnut after the combination of eight different treatment sets. Collectively the study resulted in the increase in yield production, height increase, pods and nodules

production increasing and biomass yields production after the treatment of plant with treatment combination of T₇ i.e., Brady-2 (*Bradyrhizobium* Isolates-2) + PSM with respect to the changes compared to control treatment. It can be stated that the treatment of Brady-2 (*Bradyrhizobium* isolates-2) + PSM to groundnut can be used to increase its agricultural production need. Nevertheless, the current comprehensive may also be applied to other agroecological zones to study the behaviour of microbes at different environments along with different textured soils.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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