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Effect of Different Bio-inoculants with Varied Levels of Phosphorus on Growth and Yield Attributes of Chickpea (*Cicer arietinum* L.)

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ABSTRACT

A field experiment was conducted during the *rabi* season of 2022-23 at the Research Farm of the Department of Agronomy, School of Agriculture, Lovely Professional University, Phagwara (Punjab). The experiment followed a randomized block design (RBD) with 10 treatments replicated thrice. The treatments consisted of T_1 (control), T_2 (rhizobium inoculant), T_3 (75% phosphorus+rhizobium), T_4 (75% phosphorus+rhizobium+pseudomonas), T_5 (75% phosphorus+rhizobium+pseudomonas+trichoderma), T_6 (trichoderma inoculant), T_7 (pseudomonas fluorescent inoculant), T_8 (100% phosphorus+rhizobium), T_9 (100% phosphorus+rhizobium+pseudomonas) and T_{10} (100% phosphorus+rhizobium+pseudomonas+ trichoderma). The results indicated that the T_{10} (100% phosphorus+rhizobium+pseudomonas+trichoderma) led to significant improvement in various growth and yield attributes of chickpea. This treatment exhibited the highest plant height (53.16 cm), maximum number of branches/plant (7.7), maximum number of nodules/plant (59), dry matter accumulation (15.43), maximum number of pods/plant (62.86), maximum number of seeds/pod (1.73), higher seed index (30.37 g), higher seed (18.11 q/ha), higher straw yield (29.54 q/ha) and biological yield (47.65 q/ha). Therefore, to achieve higher yields and maximize economic benefits in chickpea production, it can be suggested that farmers adopt the combined application of *Rhizobium*, PSB and *Trichoderma* along with the 100% phosphorus for sustainable crop production.

Key words: Bio-inoculants, *Rhizobium*, PSB *Trichoderma*, chickpea, sustainable crop production

INTRODUCTION

Pulses are a significant category of field crops that serve as a valuable source of high-quality protein, serving as an alternative to cereal proteins for the majority of the vegetarian population of the country (Jegadeesan and Punniyamoorthy, 2023). These legumes are often referred to as "poor man's meat" and "rich man's vegetable", making substantial contributions to the nutritional security of the nation (Kumar *et al.*, 2022). Almost three time's higher percentage of quality protein is present in pulses when compared to cereals (Kumar *et al.*, 2022). As a great source of protein, they are a basic ingredient of many traditional kitchens among Indian vegetarians who make their more balanced and nutritious diet (Kumar, 2020).

India holds a prominent position in chickpea imports, consumption and production. Despite India's status as the largest pulse-producing nation worldwide, the growth of pulse crop production in the country has experienced a gradual pace over the years (Bisht and Kumar, 2019). Chickpea is globally a widely cultivated crop and is known for its versatility (Fikre *et al.*, 2020). One of the significant benefits of chickpea cultivation is its ability to fix atmospheric nitrogen, thus contributing to improved soil fertility. Chickpea possesses the ability to fix atmospheric nitrogen, with the potential to fix up to 140 kg N/ha, thereby meeting a significant proportion of its nitrogen needs (Dembi *et al.*, 2020). Moreover, after harvest, chickpea residues contribute a considerable amount of residual nitrogen to the soil, benefiting subsequent crops and enhancing soil health and fertility (Yengkokpam *et al.*, 2022). This natural nitrogen fixation process not only reduces the need for nitrogenous fertilizers for chickpea cultivation but also positively impacts subsequent crops, resulting in cost savings for farmers.

As chickpea is a legume, it satisfies a large part of its nitrogen requirements to about 75% through the symbiotic process of nitrogen fixation which works effectively three to four weeks after sowing (Sindhu *et al.*, 2019). However, if soils have little organic matter and a low nitrogen supply, they may require 20-30 kg/ha of nitrogen as initial doses that can satisfy plant needs before forming nodules (Alene *et al.*, 2020). Traditional methods of increasing chickpea crop yields involve the excessive use of environmentally harmful chemical fertilizers (Batra *et al.,* 2020). Chemical fertilizers like urea, DAP (di-ammonium phosphate), MOP (muriate of potash), SSP (single super phosphate), etc, supply one or more nutrients at a time, but they contain nutrients in a higher percentage than other sources of nutrients. In fact, fertilizers production and consumption have been increased many folds in the last decades but nutrient removal by crops 8-10 Mt is higher than nutrient addition through fertilizers. Despite being a crucial pulse crop for both the daily diet and the agricultural sector, chickpea productivity is quite low. This gap must be filled through bio-fertilizers and organics for the sustainable management of agro-ecosystem. The excessive use of harmful synthetic fertilizers to increase food and fiber production raises concern for the following reasons: despite having sufficient nutrients in soils relying solely on artificial fertilizers experiences reduced yields. Excessive use of synthetic fertilizers, primarily nitrogen, has negatively impacted the soil's health and productivity. The current energy crisis, rising fertilizer costs and limited financial resources of farmers have highlighted the need to explore alternative options. The low crop yield can be attributed to factors such as improper fertilizer application, weed competition, incorrect sowing techniques and inadequate seed rates. Additionally, the underutilization of beneficial biofertilizers like rhizobium, phosphate-solubilizing bacteria, vesicular-arbuscular mycorrhizal (VAM) fungi, trichoderma, as well as insufficient pest and disease control measures, further contribute to reduced crop production. Therefore, it is necessary to adopt sustainable alternatives to address these challenges.

One of the most vital essential nutrients for chickpeas is phosphorus which responded significantly to its application (Singh, 2022). Phosphorus plays a crucial role in facilitating the transfer of energy and the metabolism of proteins, contributing to enhanced root growth and early crop maturity. The application of phosphorus primarily affects the root system of plants, resulting in significant effects on their overall development and performance. It helps in the proper root development which is required by the leguminous crops (Daniel *et al.,* 2022). Furthermore, it becomes helpful for better nodule formation when applied with phosphorus-solubilizing bacteria and *Rhizobium.* After phosphorus is applied to the soil, a considerable amount of it undergoes in conversion into insoluble form. However, certain microorganisms, such as phosphate-solubilizing bacteria, possess the capability to solubilize these insoluble phosphates, making them accessible to plants (Lacava *et al.*, 2021). *Rhizobium* fixes nitrogen from the air into the soil. The rhizobium attaches itself to the root hairs of legumes, forming a symbiotic association. *Rhizobium* provides about 80 to 90% of a legume's total nitrogen needs, and it enhances grain yield by 10 to 15% in field settings (Swarnalakshmi *et al.*, 2020). *Rhizobium* culture seed inoculation is a low-cost nitrogen fertilization strategy for legume crops (Soliman, 2019).

PSB (Phosphorus solubilizing bacteria) like *Pseudomonas* and *Bacillus* increases phosphorus availability for plants by converting insoluble phosphorus in the soil to soluble phosphorus (Sarmah and Sarma, 2023). In legume crops, phosphorus application has a major impact on root development, plant growth, nutrient uptake and energy transformation. Seed inoculation with rhizobium would result in an increase in the number of branches and nodules per plant, as well as the plant's yield (Patel and Thanki, 2020).

Trichoderma spp. are widely recognized as root colonizers that contribute significantly to the growth of plants compared to untreated plants (Poveda, 2021). These beneficial fungi can be applied as seed treatments and soil inoculants, resulting in improved germination percentages and the development of enhanced root length, shoot length, dry weight, fresh weight and vigour index in chickpea.

Inoculation with bio-agents in pulses has been a long-standing practice in agriculture, continually improving over the years. The use of these inoculants offers numerous benefits, including the reduction in the need for nitrogen fertilizers. Furthermore, it enhances the nodulation process, leading to higher levels of soil nitrate even after harvesting, which can be utilized by subsequent crops. Inoculated plants exhibit a significant increase in yield for both fresh and dry fodder compared to control plants that were not inoculated. Additionally, the inoculation with bio-agents significantly augments the number of nodules in chickpea plants, thereby resulting in higher chickpea yield. These findings underscore the advantages of employing bio-agents as a sustainable approach to enhance crop productivity without relying heavily on nitrogen fertilizers.

MATERIALS AND METHODS

The field experiment was conducted during the *rabi* season of 2022-23 at the Research Farm of the Department of Agronomy, School of Agriculture, Lovely Professional University, Phagwara, Punjab. The experimental site is located at latitude of 31.243357 ^{0}N, longitude of 75.696985 ^{0}E, with an average elevation of 252 meters above mean sea level. The climate of the region is characterized as sub-tropical, with hot and humid weather in summer, cool weather in winter and scattered rainfall in July, August and September. The temperature varies significantly across seasons, and the average winter rainfall is recorded as 47.4 mm. The experiment was laid out in randomized block design (RBD) comprising 10 treatments: T_1 (control), T_2 (rhizobium inoculants), T_3 (75% phosphorus+rhizobium), T_4 (75% phosphorus+ rhizobium+pseudomonas), T_5 (75% phosphorus+rhizobium+pseudomonas+ trichoderma), T_6 (trichoderma inoculant), T_7 (pseudomonas fluorescent inoculant), T_8 (100% phosphorus+rhizobium), T_9 (100% phosphorus+ rhizobium+pseudomonas) and T_{10} (100% phosphorus+rhizobium+pseudomonas +trichoderma) replicated thrice. The soil at the experimental site was identified as sandy loam with pH (7.9), organic carbon (0.42%), available N (172 kg/ha), available P (18.5 kg/ ha) and available K (164 kg/ha). The chickpea variety Samrat (GNG 469), with a duration of 160 days, was sown in line at the experimental site. The seeds were treated with *Rhizobium* spp., *Pseudomonas fluorescens* and *Trichoderma viridae* at a rate of 2 g/kg of seeds according to the treatments applied to seeds before sowing. These bioinoculants were isolated from the farms of the Department of Agronomy, Lovely Professional University, Jalandhar, Punjab, India. After the isolation, they were cultured in the laboratory with suitable media; and future subcultured for 15 days and stored in the refrigerator at 4°C under aerobic conditions and mixed with suitable carrier materials for the seed treatment. Healthy and bold chickpea seeds were dibbled into the soil with a spacing of 30 × 10 cm. The crop was harvested at physiological maturity, approximately 150 days after sowing. Data were recorded at different intervals on different aspects of the crop. Growth and yield attributes were further subjected to statistical analysis for homogenous Duccan method.

RESULTS AND DISCUSSION

The analysis of the data in Table 1 revealed a significant increase in plant height, with the highest measurement recorded in T_{10} (100% phosphorus+rhizobium+pseudomonas+trichoderma), reaching (53.16 cm) followed by T_9 (100% phosphorus+rhizobium+pseudomonas). On the other hand, T_1 (control) exhibited the lowest height, measuring 45.70 cm. These results indicated that the combined application of 100% phosphorus and all three bioinoculants could significantly enhance the growth of chickpea plants. Phosphorus is a vital nutrient for plant development, and when combined with biofertilizers, it can effectively improve nutrient availability and enhance vegetative growth. This combination plays a crucial role in promoting root and shoot development, facilitating the uptake of nitrogen and phosphorus, and overall enhancing the growth of chickpea plants. Therefore, the incorporation of bioinoculants along with an adequate supply of phosphorus can effectively increase plant height. Notably, our findings are consistent with prior studies conducted by Firdu *et al.* (2020) and Poveda and Eugui (2022) which further support the positive influence of phosphorus and biofertilizers on the height of chickpea plants.

A significant increase in number of branches/ plant, with the highest count of (7.7) was recorded in T_{10} (100% phosphorus+rhizobium+ pseudomonas+trichoderma), followed by 6.9 in

T_9 (100% phosphorus+rhizobium+ pseudomonas), while the T_1 (control) had the lowest count 4.87 of branches (Table 1). The substantial increase in branches observed in T_{10} can be attributed due to the presence of phosphorus, which plays a crucial role in promoting leaf development and cell formation, ultimately resulting in enhanced branch growth. Furthermore, the application of biofertilizers aids in increasing the solubility of phosphorus making it more readily available to the plants. This, in turn, improved photosynthesis, metabolic activity, cell division and the formation of meristematic tissues, all of which contributed to an increase in the number of branches per plant. These findings are consistent with previous research conducted by Yadav *et al.* (2021).

The treatment T_{10} (100% phosphorus + rhizobium + pseudomonas + trichoderma) exhibited the highest number of nodules/plant, with a recorded value of 59. It was followed by T_9 (100% phosphorus + rhizobium + pseudomonas), which had a value of 54.3. On the other hand, the T_1 (control) displayed the lowest number of nodules (35.3). The observed higher count of nodules in T_{10} can be attributed to the significant role played by phosphorus in nodulation. Phosphorus is known to have a positive impact on nodulation, contributing to the formation and development of nodules in leguminous plants. Additionally, the combined inoculation of phosphate solubilizing bacteria (PSB), trichoderma and rhizobium resulted in a synergistic effect among these microorganisms, further enhancing the process of nodule formation. These findings align with previous studies conducted by Schwember *et al.* (2019), Sharma *et al.* (2019) and Alamzeb and Inamullah (2023), where the application of phosphorus along with bioinoculants demonstrated an increase in nodulation and improved root proliferation in various plant species.

A significant increase in dry matter accumulation, with the highest count of (15.43) was recorded in T_{10} (100% phosphorus + rhizobium + pseudomonas + trichoderma), followed by 15.23 in T_9 (100% phosphorus + rhizobium + pseudomonas), while the T_1 (control) had the lowest count of (11.01) dry matter accumulation. The significant increase in dry matter observed in treatment T_{10} can be attributed to the combined application of three bio-inoculants, namely, rhizobium, phosphate solubilizing bacteria (PSB) and 100% phosphorus. Rhizobium plays a vital role in boosting dry matter production through the activity of the enzyme nitrogenase, which facilitates nitrogen fixation and enhances nitrogen availability for plant growth. PSB, on the other hand, contributes to increased dry matter production by improving the availability of phosphates to the chickpea plants by solubilizing phosphates, Furthermore, the application of 100% phosphorus independently contributes to the observed increase in dry matter. Phosphorus is involved in vital physiological processes within plants, including energy production through respiration, as well as cell elongation, cell division and the activation of amino acids. By ensuring an ample supply of phosphorus,

Table 1. Effect of different bioinoculants with varied levels of phosphorus on growth parameters of chickpea at 120 days

Treatment combinations	Plant height (cm)	No. of branches/ plant	No. of nodules/ plant	Dry matter accumulation (g/plant)
T_1	$45.70^{h}\pm0.3$	$4.9^{g}\pm0.09$	$35.3^{e}\pm4.49$	$11.01^{i}\pm0.085$
T_2	$50.56^{g}\pm0.3$	$5.8^{d}\pm0.16$	$45.7^{bcd}\pm5.73$	$13.04^{g}\pm0.053$
T_3	$51.34^{ef}\pm0.1$	$5.9^{d}\pm0.09$	$48.0^{bcd}\pm3.74$	$13.16^{g}\pm0.112$
T_4	$52.46^{d}\pm0.08$	$6.5^{c}\pm0.09$	$50.3^{abc}\pm5.73$	$14.42^{e}\pm0.067$
T_5	$52.55^{cd}\pm0.03$	$6.7^{b}\pm0.09$	$51.0^{ab}\pm2.44$	$15.00^{c}\pm0.069$
T_6	$51.62^{e}\pm0.2$	$5.2^{f}\pm0.16$	$39.0^{de}\pm2.94$	$14.54^{e}\pm0.067$
T_7	$51.03^{f}\pm0.06$	$5.5^{e}\pm0.09$	$41.3^{cde}\pm2.86$	$14.26^{f}\pm0.082$
T_8	$52.80^{bc}\pm0.1$	$5.9^{d}\pm0.09$	$48.7^{bc}\pm2.62$	$14.75^{d}\pm0.049$
T_9	$52.98^{ab}\pm0.0$	$6.9^{b}\pm0.09$	$54.3^{ab}\pm4.18$	$15.23^{b}\pm0.074$
T_{10}	$53.16^{a}\pm0.02$	$7.7^{a}\pm0.094$	$59.0^{a}\pm3.74$	$15.43^{a}\pm0.0805$

T_1: Control (RDF 100%), T_2: Rhizobium inoculants, T_3: 75% phosphorus + rhizobium, T_4: 75% phosphorus + rhizobium + pseudomonas, T_5: 75% phosphorus + rhizobium + pseudomonas + trichoderma, T_6: Trichoderma inoculant, T_7: Pseudomonas inoculant, T_8: 100% phosphorus + rhizobium inoculant, T_9: 100% phosphorus + rhizobium + pseudomonas inoculant and T_{10}: 100% phosphorus + rhizobium + pseudomonas + trichoderma.

all these metabolic processes are optimized, leading to improved growth attributes and a higher accumulation of dry matter in the chickpea plants. These findings align with previous studies conducted by Kumar and Singh (2023).

The significantly highest number of pods/plant (62.86) was recorded in T_{10} (100% phosphorus + rhizobium + pseudomonas + trichoderma) followed by 62.53 in T_9 (100% phosphorus + rhizobium + pseudomonas), while the lowest was observed in T_1 (control) (56.8) pods/plant (Table 2). Similarly, the significantly maximum seed index (30.37 g) was recorded in T_{10} followed by 30.11 in T_9, lowest being observed in T_1 (control) (27.83). The improved performance of yield attributes in treatment T_{10} can be attributed to the combined inoculation of three bio-inoculants along with the application of phosphatic fertilizer. This combination enhances biological nitrogen fixation and supports various physiological processes in chickpea plants. The provision of adequate nitrogen, phosphorus and other essential nutrients contributes to increased growth parameters and a higher number of pods/plant, ultimately leading to an increase in economic yield. The inclusion of trichoderma in the bio-inoculants contributes to increased vegetative growth in the chickpea plants. This positive influence on growth attributes has been supported by previous studies conducted by Singh *et al.* (2022) and Kumar and Singh (2023).

The highest grain yield (18.11 q/ha), straw yield (29.54 q/ha) and biological yield (47.65 q/ha) were recorded in T_{10} (100% phosphorus + rhizobium + pseudomonas + trichoderma) followed by T_9 (100% phosphorus + rhizobium + pseudomonas) with 17.86, 29.22 and 47.08 q/ha, respectively, while the control (T_1) displayed the lowest values of 15.26, 26.56 and 41.82 q/ha for grain, straw and biological yield, respectively (Table 3). The substantial increase in yield attributes can be attributed to the cumulative effect of the bio-inoculants *Rhizobium*, PSB and *Trichoderma* spp. These bioinoculants contribute to enhanced nutrient availability, bio-control activity against soil-borne pathogens and improved plant growth, ultimately leading to increased yield in chickpea. *Rhizobium*, through its nitrogen-fixing capabilities, enhances the supply of nitrogen to plants. This increased nitrogen availability promotes better plant growth and development, which positively impacts yield. PSB, on the other hand, plays a crucial role in solubilizing of phosphorus to enhance various metabolic processes and nutrient uptake, further contributing to increased yield. The presence of *Trichoderma* spp. in the bio-inoculants offers bio-control activity against soil-borne pathogens. By effectively suppressing the pathogenic microorganisms generally *Trichoderma* spp. creates a healthier growing environment in the soil for sustainable crop production. This protection against diseases and infections allows plants to allocate their resources towards growth and yield production, resulting in improved crop productivity. These findings are consistent with previous studies conducted by Singh (2022) and Kumar and Singh (2023).

Inoculation of all three bio-inoculants along with phosphatic fertilizers provided balanced

Table 2. Effect of different bioinoculants with varied levels of phosphorus on yield attributes of chickpea

Treatment combinations	No. of pods/ plant	No. of seeds/ pod	Seed index (g)
T_1	56.8^{f}±0.16	1.13^{e}±0.09	27.83^{f}±0.06
T_2	57.73^{e}±0.24	1.2^{de}±0.09	28.23^{e}±0.11
T_3	58.13^{de}±0.33	1.33^{cde}±0.09	28.47^{de}±0.09
T_4	61.86^{c}±0.18	1.4^{cd}±0.00	29.02^{c}±0.31
T_5	62.26^{bc}±0.18	1.53^{abc}±0.09	29.59^{b}±0.15
T_6	58.33^{de}±0.18	1.26^{de}±0.09	28.53^{de}±0.13
T_7	58^{e}±0.48	1.46^{bcd}±0.09	28.67^{d}±0.16
T_8	58.6^{d}±0.33	1.53^{abc}±0.09	29.37^{b}±0.20
T_9	62.53^{ab}±0.24	1.66^{ab}±0.09	30.11^{a}±0.14
T_{10}	62.86^{a}±0.09	1.73^{a}±0.09	30.37^{a}±0.13

Treatment details are given in Table 1.

Table 3. Effect of different bioinoculants with varied levels of phosphorus on yield of chickpea

Treatment combinations	Straw yield (q/ha)	Grain yield (q/ha)	Biological yield (q/ha)
T_1	26.56^{d}±0.392	15.26^{f}±0.165	41.82^{f}±0.208
T_2	28.54^{c}±0.254	16.62^{e}±0.192	45.16^{e}±0.193
T_3	28.88^{bc}±0.167	17.21^{d}±0.164	46.09^{d}±0.184
T_4	29.06^{ab}±0.235	17.43^{cd}±0.175	46.49^{c}±0.186
T_5	29.32^{ab}±0.142	17.71^{bc}±0.143	47.03^{b}±0.117
T_6	28.45^{c}±0.233	16.43^{e}±0.147	44.88^{e}±0.180
T_7	28.50^{c}±0.163	16.52^{e}±0.168	45.02^{e}±0.145
T_8	28.88^{bc}±0.246	17.49^{bcd}±0.139	46.37^{cd}±0.143
T_9	29.22^{ab}±0.215	17.86^{ab}±0.226	47.08^{b}±0.120
T_{10}	29.54^{a}±0.188	18.11^{a}±0.141	47.65^{a}±0.170

Treatment details are given in Table 1.

nutrition to the chickpea crop. This balanced nutrition resulted in a higher seed yield. Adequate amounts of nitrogen, phosphorus and other major nutrients provided to the plants contribute to increased growth parameters and the number of pods per plant ultimately led to increase in economic yield.

CONCLUSION

Based on the results of this study, it can be concluded that the combined application of 100% phosphorus + rhizobium + pseudomonas + trichoderma had a significant positive effect on various growth and yield parameters in chickpea cultivation. These parameters included plant height, number of branches/ plant, number of nodules/plant, dry matter accumulation, number of pods/plant, number of seeds/pod, seed index, grain yield, straw yield and biological yield. Among the different treatments evaluated T_{10} (100% phosphorus + rhizobium + pseudomonas + trichoderma) consistently performed the best, resulting in the highest values for plant height, number of branches/plant, number of nodules/plant, dry matter accumulation, number of pods/plant, number of seeds/pod, seed index, grain, straw and biological yield. Therefore, to achieve higher yields and maximize economic benefits in chickpea production, it is recommended to adopt the combined application of *Rhizobium*, PSB and *Trichoderma* along with the 100% phosphorus.

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