Optimizing chickpea growth: A multi-faceted approach integrating phosphorus zinc and bio-fertilizer

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Abstract

Phosphorus and zinc are crucial elements for plant development, and the use of organic bio-fertilizer like PSB can enhance soil fertility and crop growth, offering potential solutions for sustainable agriculture and improving crop growth. Thus, the influence of different phosphorus levels (30, 60 and 90 kg ha^{-1}) and zinc (5, 10 and 15 kg ha^{-1}) with (+) and without (-) bio fertilizer (PSB) on chickpea was investigated in pot experiment at Nowshera Kalan Nowshera, Pakistan. During winter season 2020-21. The trial was laid out in a RCB design with three repeats. A total number of 10 treatments viz. T_1 = (control), T_2 = (30 kg P + 5 kg Zn), T_3 = (60 kg P + 5 kg Zn), T_4 = (90 kg P + 5 kg Zn), T_5 = (30 kg P + 10 kg Zn), T_6 = (60 kg P + 10 kg Zn), T_7 = (90 kg P + 10 kg Zn), T_8 = (30 kg P + 15 kg Zn), T_9 = (60 kg P + 15 kg Zn), and T_{10} = (90 kg P +15 kg Zn) were studied with (+) and without (-) PSB. The volume of each pot was 3,200 cm^3, containing 2.5 kg of soil. Parameters studied were plant height (cm), root length (cm), branches plant $^{-1}$, leaves plant $^{-1}$, roots plant $^{-1}$, SDW (g plant^{-1}), RDW (g plant^{-1}) and TDW (g plant^{-1}) at 30 and 60 days after emergence (DAE). The results revealed that at 30 DAE, PSB, P, and Zn levels had significant effects on plant height, root length, branches plant^{-1}, leaves plant^{-1}, roots plant^{-1}, SDW, RDW, and TDW of chickpea. Application of PSB increased plant height and root length, while higher levels of P and Zn also promoted taller plants and longer roots. At 60 DAE, PSB continued to positively influence plant height, root length, branches plant^{-1}, and leaves plant^{-1}. The combined application of P, Zn, and PSB further enhanced these growth parameters. Interestingly, the effects of Zn and P on SDW and RDW were observed to be more pronounced at 30 DAE than at 60 DAE, while PSB affected RDW at 60 DAE. In conclusion, the trial demonstrated that the combined use of P, Zn, and PSB positively impacted the growth and development of chickpea, indicating the potential of these bio-fertilizers in sustainable agriculture for enhancing crop yields and soil fertility.

Keywords: Phosphate solubilizing bacteria, Days after Emergence, Bio-fertilizer, Shoot dry weight, Root dry weight, Total dry weight

Introduction

Chickpea scientifically known as (*Cicer arietinum* L.) is a legume, commonly referred to as Bengal grams, that has been cultivated in both Asia and Europe since ancient times. It holds the second position, following the common bean, in terms of popularity and importance among pulses. (FAOSTAT, 2015). In the year 2017-2018, the average grain yield in Pakistan was 1250 kg ha^{-1}, while in Khyber Pakhtunkhwa, it was slightly higher at 1290 kg ha^{-1} (MNFS&R, 2018). The extensive use of inorganic fertilizers, particularly nitrogen and phosphorus, in intensive cropping systems has negatively impacted the soil (Hettiarachchi et al., 2021). Embracing an organic production system can bring numerous benefits, such as promoting biodiversity, ecosystem health, natural cycles, and biological activity in the soil (Wang et al., 2021). In sustainable agriculture, organic fertilizers have a pivotal role in improving soil health, promoting growth, and boosting grain yield. These contain essential live microorganisms that are applied to seeds, soil, and roots. These microorganisms help mobilize and improve the availability of vital nutrients, boost biological activity, and enhance the soil microflora. Ultimately, this leads to improved soil health and higher yield (Ismail et al., 2014). The utilization of bio-fertilizers today helps to limit the use of artificial/chemical fertilizers and provides a valuable tool for development under a less polluted eco-system, lowering agricultural costs, and developing crop yield by giving them readily available nutritive elements and essential substances (Anhar et al., 2020). When phosphorus is added to phosphorus-deficient soil, root growth is encouraged, and maturity is frequently accelerated. For seeds to form, there must be a significant amount of phosphorus available (Gidago et al., 2012). One of the key factors that lowers productivity and yield is phosphorus deficiency (Din et al., 2021). By employing rock phosphate (RP), the availability of phosphorus can be improved by combining organic waste and phosphate-solubilizing microorganisms (Ditta et al., 2018). Zinc is important for the release of growth hormones and chlorophyll in plants. It is a chief nutrient for the general expansion and improvement of plants. Zinc is regarded as an essential part of various enzyme systems in the context of plant metabolism. For instance, it is a crucial component of carbonic anhydrase, which enables the efficient transport and utilization of CO_2 during photosynthesis (Kuldeep et al., 2018). Additionally, zinc helps to maintain a stable PH level within the cytoplasm of plant cells. In addition to preserving soil fertility, using effective nutrient management techniques in combination with helpful microbes like phosphate solubilizing bacteria (PSB) enhances chickpea growth (Singh, 2014)

Materials and Methods

The response of chickpea (*Cicer arieinum* L.) to different levels of phosphorus (30, 60 and 90 kg ha^{-1}) and zinc (5, 10 and 15 kg ha^{-1}) with (+) and without (-) bio-fertilizers (PSB) was investigated in pots experiment at Nowshera Kalan Nowshera, during winter season 2020-21. The chickpea variety used was $karak^{-1}$ obtained from the department of plant breeding and genetics. The trial was laid out in RCB design with three repeats. A total number of 10 treatments viz. T_1 = (control), T_2 = (30 kg P + 5 kg Zn), T_3 = (60 kg P + 5 kg Zn), T_4 = (90 kg P + 5 kg Zn), T_5 = (30 kg P + 10 kg Zn), T_6 = (60 kg P + 10 kg Zn), T_7 = (90 kg P + 10 kg Zn), T_8 = (30 kg P + 15 kg Zn), T_9 = (60 kg P + 15 kg Zn), and T_{10} = (90 kg P +15 kg Zn) were studied with (+) and without (-) phosphate solubilizing bacteria (PSB). The volume of each pot was 3200 cm^3 containing 2.5 kg soil per pot. The whole experiment consisted of 60 pots i.e. 30 pots (10 treatments and 3 repeats) with (+) beneficial microbes, and 30 pots without (-) beneficial microbes (10 treatments and 3 repeats). Data were gathered at two growth stages: 30 and 60 days after emergence (DAE), focusing on various plant parameters. Plant height was measured for three plants at each stage by recording the distance from the ground to the base of the highest fully expanded leaf using a measuring tape, and the average height was calculated. Similarly, root length was measured for three plants at both growth stages with the aid of a matric tape, and the average root length was computed. Branches plant-^{1}, leaves plant-^{1}, and roots plant-^{1} were manually counted in each pot at 30 and 60 DAE, and averages were determined. To assess the shoot and root weights, three plants from each stage were dried at 80°C for 24 hours and then weighed using an electronic balance. Total dry weight $plant^{-1}$ was computed by adding the root dry weight and shoot dry weight. PSB solution was dissolved in water then seeds were dipped into the solution and left for at least 5-10 minutes. Finally, eight seeds were planted in each pot. After the emergence six plants were maintained in each pot. Rock phosphate and zinc sulphate served as a phosphorus and zinc source.

Results and Discussion

Plant height (cm)

After conducting the statistical analysis, it was observed that PSB, P, and Zn levels had a significant impact on plant height at 30 days after emergence (Table 1). The utilization of PSB resulted in a growth enhancement of plants, with a recorded height of 29.2 cm compared to the PSB-free pots with a height of 28.8 cm. Regarding different levels of phosphorus, the tallest plants (29.7 cm) were observed with 90 kg P ha^{-1}, in succession to that, the plants treated with 60 kg P ha^{-1} attained a height of 29.1 cm, while the shortest plants (28.1 cm) were found with 30 kg P ha^{-1}. Among various Zn levels, 15 kg Zn ha^{-1} led to the maximum plant height (29.5 cm), followed by 10 kg Zn ha^{-1} (28.9 cm), and the smallest plants (28.4 cm) were seen with 5 kg Zn ha^{-1}. As the plants aged to 60 DAE, those treated with PSB displayed increased height (33 cm) compared to the control group (31 cm). The study's findings indicated that plant height reached 34 cm with the application of 90 kg P ha^{-1}, in contrast to 32 cm with 60 kg P ha^{-1}, and 30 cm with 30 kg P ha^{-1}. For Zn levels, the plants' tallest height (33 cm) was witnessed with the employment of 15 kg Zn ha^{-1}, with the next height achieved using 10 kg Zn ha^{-1} (32 cm), while the lowest height was recorded when 5 kg Zn ha^{-1} was used (31 cm). Plant height plays a pivotal role in growth as it is a trait controlled by genetics, but its development is also markedly affected by environmental factors, nutrient availability, and management approaches (Achieng et al., 2010): (Ashok et al. 2005) claimed that the plots receiving 60 kg P and 15 kg Zn ha^{-1} exhibited the maximum plant height. These findings align with that of Turk et al. (2002) who noticed an increase in plant height with higher P supplementation. When comparing treatments with and without PSB, it was noted that the inoculation of PSB along with any fertilizer source resulted in a plant height increase ranging from 0.1 to 1.9 cm (Song et al., 2000). It is suggested that the higher availability of nutrients might have promoted cell division, contributing to the development of taller plants (Iqtidar et al., 2006).

Root length (cm)

At 30 days after emergence, PSB significantly increased of root length (20.2 cm) whereas, no PSB application recorded minimum length (19.4 cm). An increased was seen up to 20.6 cm with 90 P kg ha^{-1}, when contrasted with 60 kg P ha^{-1} (19.9 cm) and 30 kg P ha^{-1} (18.9 cm). For Zn levels, 15 kg Zn ha^{-1} produced maximum value of root length (20.3 cm), followed by 5 kg Zn ha^{-1} (19.8 cm) and minimum value recorded was on 10 kg Zn kg ha^{-1} produced (19.2 cm). Data recorded at 60 DAE revealed that, application of PSB significantly increased root length (23 cm) compared to no PSB pots (21 cm). Root length exhibited variations among different P levels, reaching up to 25 cm with 90 P kg ha^{-1}, succeeded by 60 kg P ha^{-1} with a length of 21 cm, while the lowest measurement of 20 cm was recorded with 30 kg P ha^{-1}. Moreover, it was found that applying 15 kg Zn ha^{-1} led to the most significant root length of 23 cm. Subsequently, the root length was 22 cm when 10 kg Zn ha^{-1} was applied, and 21 cm when 5 kg Zn ha^{-1} was used. Research conducted by Gulut and Ozdemir (2021), it was observed that certain chickpea genotypes experienced an increase in specific root length when exposed to phosphorus (P). Similarly, another study undertaken by Ullah et al. (2019) observed that the utilisation of zinc (Zn) resulted in an increased root length in chickpea plants. Furthermore, Zhang et al. (2020) stated that the presence of beneficial microbes had a positive impact on root length and volume in plants. Additionally, Shah et al. (2018) discovered that organic fertilizers contributed to the improvement of soil physical conditions. This was achieved through the promotion of soil porosity and the reduction of bulk density, creating an optimal environment for seamless root penetration and growth within the soil.

Number of branches $plant^{-1}$

Branch $plant^{-1}$ at 30 DAE was significantly impacted by P and Zn levels. It was determined that PSB had no substantial impact. 90 P kg ha^{-1} increased the number of branches (12.9) when compared to 60 P kg ha^{-1} (11.2) and 30 P kg ha^{-1} (9.9). The highest number of branches were produced by Zn levels at 15 kg Zn kg ha^{-1} (11.8), followed by 10 kg Zn kg ha^{-1} (11.2) and fewer at 5 kg Zn kg ha^{-1} (10.9). Interactive effect of BM x ZN x P was found significant. Further data, at 60 DAE showed that PSB increased number of branches $plant^{-1}$ (24) in comparison with no PSB applied (20). Branches $plant^{-1}$ increased up to 24 with 90 P kg ha^{-1}, when related to 60 kg P ha^{-1} (23)

and 30 kg P ha^{1} (19). Moreover, application of 15 kg Zn kg ha^{-1} had the highest branches plant^{-1} (25), followed by 10 kg Zn ha^{-1} (23) and the less branches (18) was recorded with 5 kg Zn ha^{-1}. Singh and Singh, (2014) reported that P and Zn application increased branches plant^{-1} in chickpea. Nka et al. (2014) reported that phosphorus fertilizer application to pea varieties had positive effect on branches per plant. Moreover, work done by Susilowati et al. (2022) revealed that seed priming with PSB has a positive impact on growth parameters.

Number of leaves plant^{-1}

Data pertaining to the leaves plant^{-1} showed that PSB and P levels had a significant impact at 30 days after emergence (DAE), whereas Zn levels did not show any significant effect. The highest number of leaves (161) was observed in plants treated with PSB, while the control group had a lower leaf count (151). The number of leaves increased up to 159 with the application of 90 P kg ha^{-1}, as compared to 60 kg P ha^{-1} (157), and the lowest leaf count (152) was reported in plots that received 30 kg P ha^{-1}. At 60 DAE, the plants inoculated with PSB exhibited an increased number of leaves (193) compared to those without PSB (160). Similarly, the number of leaves increased up to 193 plants^{-1} with the application of 90 P kg ha^{-1}, as compared to 60 kg P ha^{-1} (160) and 30 kg P ha^{-1} (147). Regarding Zn levels, a significant difference was observed, where 15 kg Zn ha^{-1} resulted in the highest number of leaves (180), followed by 10 kg Zn ha^{-1} (165), and the lowest was noted for 5 kg Zn ha^{-1} (155). These statements are in agreement with the results reported by Singh et al. (2018), who observed an 8.12 percent increase in leaves plant^{-1} with the application of 60 kg P_2O_5 ha^{-1} compared to 30 kg P_2O_5 ha^{-1} and a 27.28 percent increase over the control. Similarly, Janmo hammadi et al. (2018) found that PSB treatment combined with Zn led to an enhanced bioavailability of micronutrients, thereby affecting the growth attributes.

Number of roots plant $^{-1}$

Roots plant^{-1} at 30 DAE was significantly affected (Table 2). Application of PSB significantly increased number of Roots (22) in comparison with pots receiving no PSB (18). Among P levels number of roots increased up to (22) with 90 P kg ha^{-1}, when compared with 60 kg P ha^{-1} (20) and 30 kg P ha^{-1} (17). For Zn levels, 15 kg Zn kg ha^{-1} recorded more roots (21 roots plant^{-1}), succeeded by 10 kg Zn ha^{-1} (20) whereas, less roots were recorded for 5 kg Zn ha^{-1} (18). Similarly, at 60 DAE, application of PSB significantly increased number of roots (27) in comparison with plants receiving no

PSB (23). Among P number of roots increased up to 26 roots with fertilization of 90 P kg ha^{-1}, when compared with 60 kg P ha^{-1} (25) and 30 kg P ha^{-1} (23). Moreover 15 kg Zn kg ha^{-1} produced maximum number of roots (26), followed by 10 kg Zn ha^{-1} (25) and less roots were reported for 5 kg Zn ha^{-1} (24). Zinc-containing phosphorus (P) fertilizers increased shoot growth and also showed increases in root growth (Crop nutrition, 2023). While there is no direct evidence of PSB application increasing the number of roots in chickpea, the improved growth and yield attributes suggest that it may have a positive impact on root growth. The statement can be supported by the research conducted by Katiyar et al. (2020), wherein they reported that the application of PSB has been observed to enhance both growth and yield characteristics of chickpea.

Shoot dry weight (g $plant^{-1}$)

Analysis of data clarified that at 30 DAE P and PSB had no significant effect on SDW, However Zn had a significant effect. Employment of 10 kg Zn ha^{-1} produced highest SDW (1.38 g) as compared to other two levels of Zn. Furthermore, no significant effect of Zn and P on SDW at 60 DAE was noted. However, PSB treatment was significant. It affected SDW (2.4 g) as compared to no PSB application (2.2 g). The interaction revealed that SDW increased with combined use of P, Zn and PSB than without PSB and the increase was higher when both P and Zn levels increased. According to Ullah et al. (2019), their study revealed that providing sufficient zinc supply to chickpea plants improved their ability to withstand drought and heat stresses, resulting improved SDW. Additionally, introduction of phosphate solubilizing bacterial strains positively influenced the shoot length and shoot dry weight of chickpea, as reported by Gul et al. (2004).

Root dry weight (g $plant^{-1}$)

Analysis of the data showed that P and Zn considerably altered RDW, whereas PSB treatments showed no significant effect. Compared to 60 kg ha^{-1} (0.31 g) and 30 kg ha^{-1} (0.3 g), 90 kg P ha^{-1} yielded higher RDW (0.33 g). The maximum RDW (0.35 g) was obtained by the application of 10 kg Zn ha-^{1} compared to the other two Zn levels (0.32 g and 0.26 g, respectively). Further analysis of the data at 60 DAE clarified that RDW was affected by P levels, Zn levels and PSB treatment. For P levels 90 kg P ha^{-1} produced higher RDW (0.41g) than 60 kg ha^{-1} (0.36 g) and 30 kg ha^{-1} (0.36 g). Both 15 kg ha^{-1} Zn and 10 kg ha^{-1} Zn produce same RDW (0.39 g each) in comparison with 5 kg

Zn ha^{-1} (0.34 g). P x Zn x PSB interaction revealed that RDW increased with combined use of P, Zn and PSB and the increase was higher when both P and Zn levels increased. Elevating the levels of phosphorus (P) in the soil solution can lead to an increased diffusion of Pi (phosphate) towards the roots, thereby influencing root development, as reported by Lambers et al. (2006). Similarly, the trial carried out by Valenciano et al. (2011) indicated that the introduction of zinc resulted in a notable improvement in RDW of chickpea. Likewise, another study demonstrated that the RDW was improved for all treated pots. The most substantial increase in root dry weight was observed in specific combinations of PSB (phosphate-solubilizing bacterial) strains, as highlighted in the study by Benjelloun et al. (2021).

Total dry weight (g $plant^{-1}$)

P and Zn levels influenced TDW. However, no effect was noted by PSB treatment at 30 DAE. 90 kg P ha^{-1} produced higher TWD (01.66 g) than 60 kg ha^{-1} (1.64 g) and 30 kg ha^{-1} (1.61 g). The data also showed that application of 15 kg Zn ha^{-1} reported the highest TDW (0.74 g) as compared to 10 kg ha^{-1} Zn and 5 kg ha^{-1} Zn (1.70 g and 1.51 g, respectively). At 60 DAE, PSB treated pots produced higher TDW (2.80 g) than without PSB application (2.5 g). 90 kg P ha^{-1} produced higher TDW (3.0 g) than 60 kg ha^{-1} (2.8 g) and 30 kg ha^{-1} (2.7 g). However, there were no significant effect with the application of Zn on TDW. Our findings are aligning with those of Singh et al. (2018), who observed that the dry weight per plant increased as the crop aged, reaching its peak at the harvest stage. Additionally, several other studies by Ramesh et al. (2011) and Mishra and Jain (2013), reported a similar trend of increased dry matter when bio-fertilizers were applied.

Table 1: Effect of phosphorous and zinc levels (kg ha^{-1}) with and without PSB on Plant height (cm), Root length (cm), Number of branches plant^{-1} and Number of leaves plant^{-1} of chickpea at 30 and 60 DAE.

Treatment	Plant height (cm)		Root length (cm)		Number of branches plant^{-1}		Number of leaves plant^{-1}	
	At 30 DAE	At 60 DAE	At 30 DAE	At 60 DAE	At 30 DAE	At 60 DAE	At 30 DAE	At 60 DAE
Phosphorus (kg ha^{-1})								
30	28.1c	30c	18.9c	20c	9.9c	19c	152c	147c
60	29.1b	32b	19.9b	21b	11.2b	23b	157b	160b
90	29.7a	34a	20.6a	25a	12.9a	24a	159a	193a
LSD $_{(0.05)}$	0.17	0.25	0.18	0.20	0.22	0.48	1.52	5.67
Zinc (kg ha^{-1})								
5	28.4c	31c	19.2c	21c	10.9c	18c	152	155c
10	28.9b	32b	19.8b	22b	11.2b	23b	158	165b
15	29.5a	33a	20.3a	23a	11.8a	25a	158	180a
LSD $_{(0.05)}$	0.17	0.25	0.18	0.20	0.22	0.48	ns	5.67
Bio-fertilizer (PSB)								
With (+)	29.2 a	33a	20.2a	23a	11.6	24a	161a	182a
Without (-)	28.8 b	31b	19.4b	21b	11.0	20b	151b	151b
LSD $_{(0.05)}$	0.34	1.03	0.40	0.92	ns	3.99	4.78	22.72
Interaction	**	ns	*	**	*	*	ns	ns
CV %	7.75	9.78	5.67	5.08	6.14	7.08	3.07	4.84

Mean in columns followed with the same letters are non-significant at P-value = 5 %

Table 2: Effect of phosphorous and zinc levels (kg ha^{-1}) with and without PSB on Number of roots plant^{-1}, Shoot dry weight (g plant^{-1}), Root dry weight (g plant^{-1}) and Total dry weight (g plant^{-1}) of chickpea at 30 and 60 DAE.

Treatment	**Number of roots plant^{-1}**		**Shoot dry weight (g plant^{-1})**		**Root dry weight (g plant^{-1})**		**Total dry weight (g plant^{-1})**	
	At 30 DAE	At 60 DAE	At 30 DAE	At 60 DAE	At 30 DAE	At 60 DAE	At 30 DAE	At 60 DAE
Phosphorus (kg ha^{-1})								
30	17c	23c	1.31	2.3	0.30c	0.34c	1.61c	2.7c
60	20b	25b	1.33	2.4	0.31b	0.36b	1.64b	2.8b
90	22a	26a	1.33	2.4	0.33a	0.41a	1.66a	3.0a
LSD $_{(0.05)}$	0.54	0.41	ns	ns	0.01	0.01	0.01	0.02
Zinc (kg ha^{-1})								
5	18c	24c	1.25c	2.4	0.26c	0.33b	1.51c	2.7
10	20b	25b	1.38a	2.4	0.32b	0.39a	1.70b	2.7
15	21a	26a	1.35b	2.3	0.35a	0.39a	1.74a	2.7
LSD $_{(0.05)}$	0.54	0.41	0.01	ns	0.01	0.01	0.01	ns
Bio-fertilizer (PSB)								
With (+)	22a	27a	1.33	2.4a	0.32a	0.40a	1.65	2.8a
Without (-)	18b	23b	1.32	2.2b	0.30a	0.34b	1.62	2.5b
LSD $_{(0.05)}$	2.84	1.32	ns	0.02	ns	0.03	ns	0.03
Interaction	ns	ns	ns	**	ns	**	ns	ns
CV %	8.66	5.12	10.88	6.17	9.46	5.29	7.97	7.75

Mean in columns followed with the same letters are non-significant at P-value = 5 %.

Conclusion

In conclusion, the study investigating the response of chickpea to varying levels of phosphorus and zinc with and without bio-fertilizer (PSB) revealed significant positive effects on plant height, root length, branches, leaves, number of roots, SDW, RDW and TDW of chickpea at both 30 and 60 days after emergence (DAE). The application of PSB, phosphorus, and zinc positively influenced plant growth and development, with higher levels of these nutrients promoting taller plants and longer roots. The combined use of phosphorus, zinc, and PSB further enhanced these growth parameters, indicating the potential of these bio-fertilizers in sustainable agriculture for improving crop yields and soil fertility.

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