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Optimizing P and Zn Levels for Greengram Growth: Insights into P-Zn Interaction and Rhizospheric Soil pH

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Authors' contributions

This work was carried out in collaboration among all authors. Author SMA designed the study, performed the statistical analysis, wrote the protocol, and drafted the initial manuscript. Authors AS and SAP managed the analyses of the study and provided critical revisions to the manuscript. Authors PB, SS and SP conducted the literature searches and contributed to the interpretation of the results. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Phosphorus (P) and zinc (Zn) are essential nutrients for plant growth, and their interaction can significantly influence nutrient uptake and crop productivity. Understanding the optimal balance between P and Zn is crucial for efficient fertilization strategies, especially in soils with low P status. This study aimed to investigate the impact of P-Zn interaction on greengram growth and its relationship with rhizospheric soil pH.

Materials and Methods: A pot experiment was conducted using red and lateritic soil, with 21 treatments consisting of different levels of P and Zn. Greengram (*Vigna radiata* L.) plants were subjected to these treatments, and after 28 days, measurements were taken for dry weight, P and Zn content in shoots, and rhizospheric soil pH.

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Results: After 28 days of seeding, strong P-Zn interactions were observed in the soil-plant system, significantly affecting greengram growth, as well as Zn and P accumulation, and rhizospheric soil pH. High P addition resulted in a significant decrease in Zn content, while the dosage of Zn had minimal impact on P content. Interestingly, Zn treatment without P addition did not enhance dry matter. However, increasing Zn levels with P addition up to 200 mg kg-1 soil improved crop growth. Beyond this threshold, yields started to decline, potentially due to P toxicity and/or Zn deficiency. Surprisingly, excessive inorganic P addition increased the rhizospheric soil pH, which could potentially reduce Zn uptake and subsequently elevate the P-Zn molar ratio in the shoot.

Conclusion: This study demonstrates that understanding the optimal doses of P and Zn and their influence on nutrient absorption and rhizospheric soil pH is crucial for sustainable crop production. The findings provide valuable insights for designing effective fertilization strategies in P-deficient acid soils to improve greengram productivity while maintaining nutrient balance.

Keywords: Greengram; interaction; molar ratio; phosphorus; rhizospheric; zinc.

1. INTRODUCTION

Pulses play a crucial role in productive agriculture, particularly after cereals. Mungbean (*Vigna radiata* L.), also known as green gram, is a resilient and short-duration pulse crop. In terms of cultivation area and production in India, it ranks third among edible pulses, trailing behind chickpea (*Cicer arietinum*) and pigeon pea (*Cajanus cajan*) [1]. In India, mungbean is cultivated across approximately 4 million hectares of land, but its average yield of 474 kg/ha falls significantly below its productive potential [2]. Achieving high yields necessitates a balanced supply of macro and micronutrients [3]. Phosphorus (P) and zinc (Zn) are two essential elements required in appropriate quantities, especially for pulse crops. However, these minerals often exhibit adverse interactions within soil-plant systems [4,5]. Previous studies have investigated the relationship between P and Zn in plant nutrition. In some instances, the addition of P has been found to reduce overall Zn uptake by plants [6], while in other cases, it has had no effect or even increased Zn uptake [7]. Conversely, Zn application has been observed to decrease the P content in grains [8]. The excessive use of P fertilizers in Zn-deficient soils leads to a Zn shortage in plants caused by P imposition [9,10]. This adverse effect of excessive P fertilizer use can hinder global Zn biofortification efforts and ultimately impede crop growth.

Root morphology and rhizosphere processes play a vital role in Zn acquisition, as indicated by the positive correlation between Zn uptake efficiency in rice cultivars and factors like root length, root volume, and root surface area [11]. Additionally, the colonization of roots by arbuscular mycorrhizal fungi (AMF) significantly increases Zn concentrations in cereal crops, such as wheat [12]. In maize, P deficiency leads to increased organic acid exudation from roots, enhancing soil Zn availability [13]. Conversely, excessive P fertilizer application negatively affects root morphology, resulting in decreased root dry weight, length, surface area, and influencing processes like AMF colonization and root exudation [14]. Moreover, higher P addition can alter the pH of the surrounding rhizospheric soil, which is closely associated with the bioavailability of elements such as P and Zn [15]. Phosphate adsorption tends to increase soil pH at low levels [16]. Notably, plants grown in acidic soils exhibit higher concentrations of Zn [17]. Given the need for significant P inputs in low-P soils, it becomes crucial to determine the optimal dosage of P and Zn, as exceeding this level can negatively impact root and rhizosphere properties [18]. Limited research has focused on the interaction between P and Zn, particularly in relation to rhizospheric soil pH, especially in low-P soil conditions. This study aims to evaluate the influence of P and Zn interaction on the growth and uptake of P and Zn in mungbean cultivated in red and lateritic soils, as well as the effects on the rhizospheric soil pH.

2. MATERIALS AND METHODS

Collection of soil samples: To conduct the experiment, soil samples were collected from the uncultivated area located at the Regional Research Station in Jhargram, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India (coordinates: 22°27’21.17”N, 87°00’42.91”E). Surface soil samples were obtained by using a spade after removing the surface vegetation cover. The collected soil samples, taken from a depth of 0-20cm, were air-dried at room temperature and passed through a 2 mm screen for further analysis. The soil in the red and lateritic soil zone was classified as a coarse loamy Typic Haplustulf. Some of the soil properties were as follows: pH 4.34 (soil:water ratio of 1:2.5), EC (electrical conductivity) 0.51 dS/m (soil:water ratio of 1:2.5), organic carbon content 3.2 g/kg, water holding capacity 27%, Bray P (5.05 mg/kg, and DTPA-extractable Zn 1.08 mg/kg [19].

Pot experiment details: The pot experiment took place at the experimental greenhouse located at Bidhan Chandra Krishi Viswavidyalaya. The experiment involved two variables: P and Zn. The soils were treated with seven different levels of P using KH_2PO_4 and were incubated at room temperature for one day. The P levels used were 0.0, 20.0, 50.0, 100.0, 200.0, 400.0, and 600 mg P kg^{-1} soil. For each P treatment, three levels of Zn in the form of $ZnSO_4.7H_2O$ were applied at rates of 0.0, 10.0, and 20.0 mg Zn kg^{-1} soil. Consequently, there were a total of 21 treatments (7 levels of P × 3 levels of Zn), and each treatment was replicated three times in the pot culture experiment. This resulted in a total of 63 pots (21 × 3).

The plants were grown in non-draining plastic bags filled with one kg of treated soil. After germination, ten greengram seeds were planted, and six healthy plants were maintained in each bag. Nutrients were supplied through a nutrient solution containing the following composition in mg kg^{-1} soil: N 100, K 100, Ca 15, Mg 6, Mn 4, Mo 1, Cu 1, and B 0.2. The nitrogen (N) was divided equally between NH_4Cl and $NaNO_3$. The experimental design employed was a Randomized Complete Block Design (RCBD). To maintain optimal moisture levels, the soils were watered daily to retain 50% of their water holding capacity.

After 28 days of seedling emergence, the stems were harvested by cutting them just above ground level. The harvested plant samples were washed with distilled water, dried at 70±2°C for 48 hours, and weighed.

P and Zn measurement: The P and Zn content in the dried plant samples were determined by digestion. The plant samples were digested using a triacid mixture consisting of nitric acid (HNO_3), sulfuric acid (H_2SO_4), and perchloric acid ($HClO_4$) in a volume ratio of 10:1:4. Subsequently, the amount of Zn in the digested extracts was measured using atomic absorption spectrophotometry (AAS) with a Perkin Elmer instrument. On the other hand, the P content was determined using the Molybdate Blue method.

pH measurement: After cutting the shoots at the soil surface, the plastic bags were lifted from the pots to separate the roots from the soil. Approximately 1.0 g of fresh fine roots with rhizospheric soil was collected and soaked in 20 mL of 0.2 mM $CaCl_2$ solution for approximately 5 minutes. The pH of the rhizospheric extract was measured using a pH meter, following the method described by He et al. [20].

Subsequently, soil samples were air-dried and passed through a 2-mm sieve. From each pot, approximately 10 g of bulk soil sample was taken to measure the soil pH. This was done by mixing the soil sample with 25 mL of 0.02 M $CaCl_2$ solution. The pH of the soil extract was then measured.

Calculation and statistical analysis: To determine the uptake of P and Zn by the greengram shoot, the dry weight of the shoot was multiplied by the concentrations of P and Zn. This calculation provided the respective P and Zn uptake values. The P/Zn molar ratio was calculated by multiplying the P/Zn content by the ratio of the molecular weight of Zn to the molecular weight of P.

The statistical analysis was performed using the general linear model in the IBM SPSS Statistics 22.0 software package. The effects of soil Zn rate, P rate, and their interaction (Zn × P) on all measured and calculated parameters were examined. Effects were considered significant if the *P*-values were less than 0.05, and trending to be significant if the p-values were between 0.05 and 0.10. Finally, the curves representing the data were generated using Sigma Plot version 10 software.

3. RESULTS

The dry matter weight of greengram shoots: The results of the study showed the impact of different levels of P and Zn on the dry weight of greengram, as illustrated in Fig. 1. The statistical analysis presented in Table 1 confirmed that the effects of P and Zn addition on greengram dry weight were significant ($P \leq 0.05$).

Inadequate P levels had a detrimental effect on crop development, with no response observed to Zn addition. The addition of P up to 200 mg kg^{-1} soil resulted in enhanced crop growth, with dry weight ranging from 31.66 to 106.47 mg $plant^{-1}$. However, when P levels exceeded 200 mg kg^{-1} soil, the yield started to decline, reaching 66.30 mg $plant^{-1}$.

Table 1. Sources of variance in different parameters under combined addition of P and Zn in greengram

	Dry weight	P conc.	P uptake	Zn conc.	Zn uptake	P/Zn molar ratio	Soil pH	Rhizosphere pH
Main effects								
P addition	2687***	48338***	7025***	8869***	1809***	49373***	133***	539***
Zn addition	1256***	0.51^{NS}	649***	69848***	10789***	47544***	3.2^{NS}	39***
Interaction								
P×Zn	73***	61***	69***	306***	230***	5468***	1.1^{NS}	4.1***

**** Significant F at P ≤ 0.05*

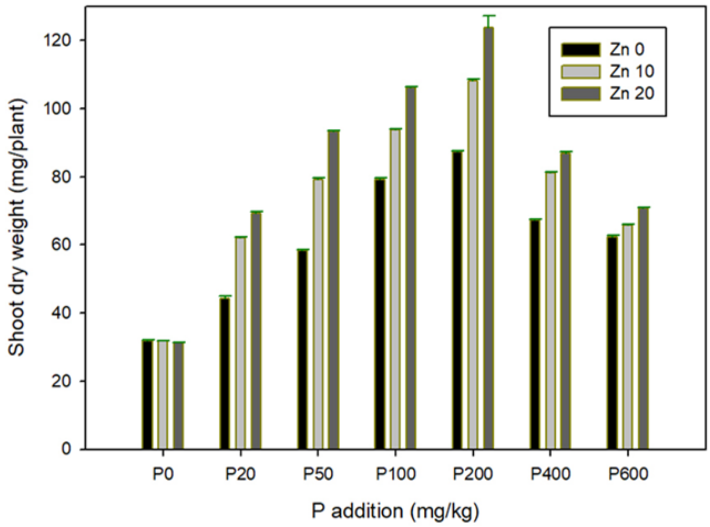

Fig. 1. Effects of different levels of P and Zn on shoot dry weight (mg plant^{-1}) of greengram. Data are presented as means ± SE (n = 3)

On the other hand, Zn treatments showed an increase in dry matter yield, ranging from 61.53 mg plant^{-1} in the Zn_0 treatment to 83.09 mg plant^{-1} in the Zn_{20} treatment. Notably, the impact of Zn addition on dry matter varied depending on the P levels, and vice versa, indicating a significant interaction between P and Zn.

Among the treatments, the highest dry matter production of 123.90 mg plant^{-1} was observed in the treatment that received 200 mg P kg^{-1} soil and 20 mg Zn kg^{-1} soil. This was followed by the treatment with 200 mg kg^{-1} P and 10 mg kg^{-1} Zn, which yielded 108.23 mg plant^{-1}. Conversely, the lowest dry matter production of 31.26 mg plant^{-1} was observed in the treatment where 20 mg Zn kg^{-1} was added without any P supplementation.

P concentration and uptake by greengram shoots: The P content in the greengram shoots was significantly influenced by P addition and also by the interaction between P and Zn ($P \leq 0.05$), as indicated in Table 1.

The addition of P had a positive effect on the P content in greengram shoots, regardless of the Zn levels (Fig. 2). P concentrations in the shoots ranged from 1.70 mg/g in the P_0Z_{20} treatment to 20.32 mg/g in the $P_{600}Z_{20}$ treatment. Interestingly, the Zn treatments did not have a noticeable impact on the P content in plant tissues when high P rates were applied. However, at low P levels, high Zn application led to a reduction in P concentration in the shoots.

Visual observations of the plants provided insights into P deficiency and toxicity symptoms. Plants that received 50 mg P/kg soil or less exhibited signs of P insufficiency, such as thin stems and narrow leaves. On the other hand, some leaves displayed symptoms of P toxicity, such as chlorosis and necrosis on the older leaf margins, when P levels exceeded 400 mg/kg soil.

The interaction between P and Zn had significant effects on P uptake by the shoots under different P and Zn rates (Fig. 2). The addition of both P and Zn significantly increased P absorption by the shoots. Plants grown in pots treated with 200 mg P kg^{-1} soil had the highest P uptake (1.68 mg plant^{-1}), while plants without any P treatment showed the lowest P uptake (0.06 mg plant^{-1}). The application of Zn treatments led to a significant increase in P absorption, with 20 mg kg^{-1} Zn being the most effective (1.03 mg plant^{-1}). In contrast, the control plants without Zn supplementation had the lowest P uptake (0.78 mg plant^{-1}). The highest P uptake was observed in the $P_{200}Zn_{20}$ treatment (1.94 mg P plant^{-1}), but as P supply increased further, there was a decline in P uptake due to poorer yield resulting from nutrient imbalance. Notably, higher P addition combined with low Zn resulted in symptoms of both P toxicity and Zn insufficiency, indicating the complex interaction between these nutrients.

Zn concentration and uptake by greengram shoots: The concentration and uptake of Zn in greengram shoots were significantly influenced by different levels of P and Zn, as well as their interaction ($P \leq 0.05$), as indicated in Table 1.

Increasing the supply of Zn resulted in higher Zn content in the plants, while Zn content declined with increasing P application (Fig. 3). The maximum Zn concentration (239.06 mg kg^{-1}) was observed when no P was applied in combination with 20 mg Zn kg^{-1} soil, while the lowest Zn concentration (50.07 mg kg^{-1}) was obtained when no Zn was used in conjunction with 600 mg kg^{-1} P. Increasing P from 0 to 600 mg kg^{-1} led to a 45.29% decrease in shoot Zn content, whereas the addition of Zn from 0 to 20 mg kg^{-1} resulted in a 133% increase.

Plants that did not receive Zn supplementation exhibited poor development and low Zn concentrations in their shoots, but they did not show severe symptoms of Zn deficiency on their leaves up to 400 mg kg^{-1} P addition. However, when P concentrations exceeded 400 mg kg^{-1}, the middle and fully grown leaves displayed symptoms of Zn deficiency, such as interveinal chlorosis and reddish-brown patches, at all Zn levels.

The addition of Zn significantly increased Zn absorption, with the highest Zn uptake (15.83 µg $plant^{-1}$) observed in the Zn_{20} treatment. The maximum amount of Zn (14.70 µg Zn $plant^{-1}$) was taken up by greengram shoots when P was applied at 200 mg P kg^{-1} soil, likely due to increased dry matter production in the shoots. This was followed by 14.38 µg Zn $plant^{-1}$ in the P_{100} treatment. All rates of Zn fertilization enhanced shoot Zn absorption with P addition up to 200 mg P kg^{-1} soil, but above this dose, Zn uptake decreased. The highest Zn uptake (21.40 µg $plant^{-1}$) was observed in the $P_{200}Z_{20}$ treatment, which was nearly identical to the $P_{100}Z_{20}$ treatment (20.90 µg $plant^{-1}$). The findings suggest that high P concentrations (>200 mg P kg^{-1}) reduce the total Zn absorption by the shoots.

P-Zn molar ratios in greengram shoots: The P-Zn molar ratio in greengram shoots, which is an important factor determining P-induced Zn deficiency and a bioavailability trait in crop growth, was significantly affected by the combined addition of P and Zn ($P \leq 0.05$), as shown in Table 1.

The P-Zn molar ratios ranged from 14.92 to 826.22 in the experimental treatments. The highest P-Zn molar ratio (824.12) was observed when no Zn was applied in combination with 600 mg P kg^{-1}, while the lowest ratio (14.87) was found when no P was applied in conjunction with 20 mg kg^{-1} Zn. The addition of Zn substantially decreased the molar ratio at different P levels, whereas the addition of P significantly increased the ratio at all Zn levels (Fig. 4).

Greengram plants exhibited better performance after 4 weeks of growth when the P-Zn molar ratio was between 130 and 180, which was achieved with P additions ranging from 100 to 200 mg kg^{-1} regardless of Zn addition. When the P-Zn molar ratio exceeded 210, both Zn deficiency and P toxicity were observed, indicating an imbalance between the two nutrients.

These findings highlight the importance of maintaining an appropriate P-Zn molar ratio in crop plants to avoid nutrient imbalances and promote optimal growth and productivity.

Soil and rhizospheric extract pH: The addition of P had a notable impact on both soil and rhizospheric pH, resulting in significant changes ($P \leq 0.05$). Conversely, the addition of Zn did not have a significant effect on soil pH, but had impact on rhizospheric pH. However, an intriguing finding emerged from the interaction between P and Zn; it exerted a significant influence on the pH of the rhizosphere (Table 1).

According to Table 2, the bulk soil pH ranged from 4.22 to 4.96, while the rhizospheric extract pH ranged from 4.71 to 6.31. In all cases, the rhizospheric extract pH was higher than the bulk soil pH, indicating a more alkaline environment in the rhizosphere. Furthermore, there was a positive correlation between the pH of the bulk soil and the rhizospheric extract, as shown in Table 3.

Among the treatments, $P_{200}Z_{20}$ had the highest pH values for both the soil (4.96) and the rhizospheric extract (6.31). On the other hand, P_0Z_{20} and P_0Z_{10} exhibited the lowest pH values for both the soil (4.22) and the rhizospheric extract (4.71). The addition of different levels of P had a significant effect on both the soil and rhizospheric extract pH ($P \leq 0.05$), as indicated in Table 1. While the rhizospheric extract pH seemed to be influenced by the interaction between P and Zn, there was no impact of Zn addition on either pH.

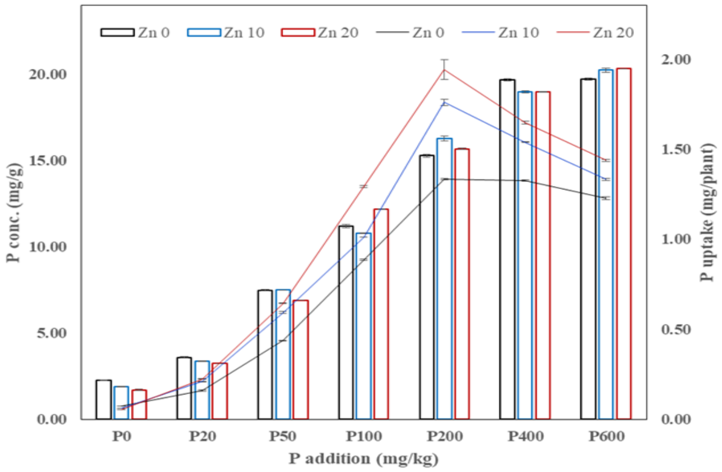

Fig. 2. Effects of different levels of P and Zn on P concentration (mg g^{-1}) and uptake (mg $plant^{-1}$) by greengram shoot. Data presented as means ± SE (n=3). Bar and line represents P conc. and uptake, respectively

Table 2. Effects of different levels of P and Zn on soil and rhizospheric extract pH

	Soil pH				**Rhizospheric extract pH**			
	Zn 0	**Zn 10**	**Zn 20**	**Mean**	**Zn 0**	**Zn 10**	**Zn 20**	**Mean**
P 0	4.23	4.22	4.22	4.22^{g*}	4.91	4.71	5.04	4.89^{g}
P 20	4.33	4.28	4.32	4.31^{f}	5.14	5.15	5.43	5.24^{f}
P 50	4.51	4.43	4.45	4.47^{e}	5.49	5.52	5.56	5.52^{e}
P 100	4.61	4.52	4.54	4.56^{d}	5.60	5.61	5.71	5.64^{d}
P 200	4.66	4.65	4.66	4.66^{c}	5.75	5.74	5.81	5.77^{c}
P 400	4.76	4.78	4.91	4.82^{b}	5.86	5.92	6.04	5.94^{b}
P 600	4.94	4.86	4.96	4.92^{a}	6.27	6.22	6.31	6.27^{a}
Mean	4.58^{b}	4.54^{ab}	4.58^{a}		5.57^{ab}	5.55^{b}	5.70^{a}	

***The means with the same letters are not significantly different at 5% level of probability*

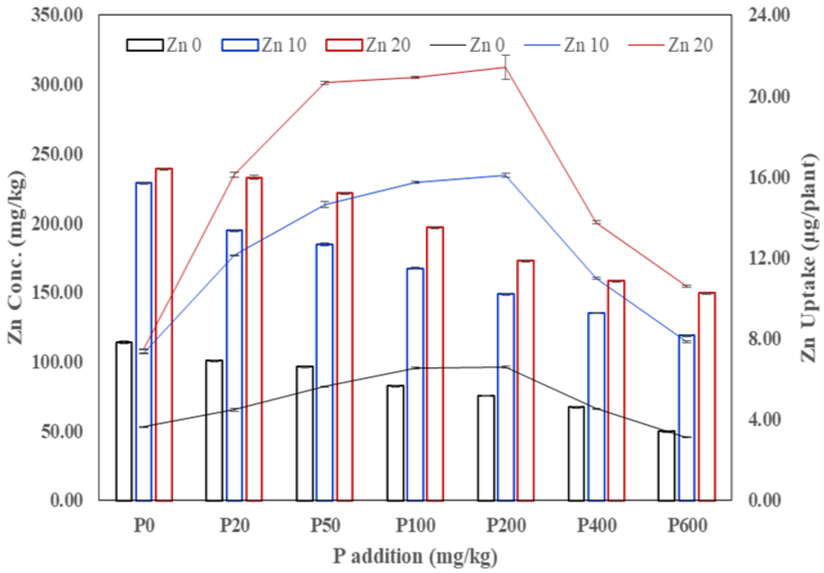

Fig. 3. Effects of different levels of P and Zn on Zn concentration (mg kg^{-1}) and uptake (µg $plant^{-1}$) by greengram shoots. Data presented as means ± SE (n=3). Bar and line represent Zn conc. and uptake, respectively

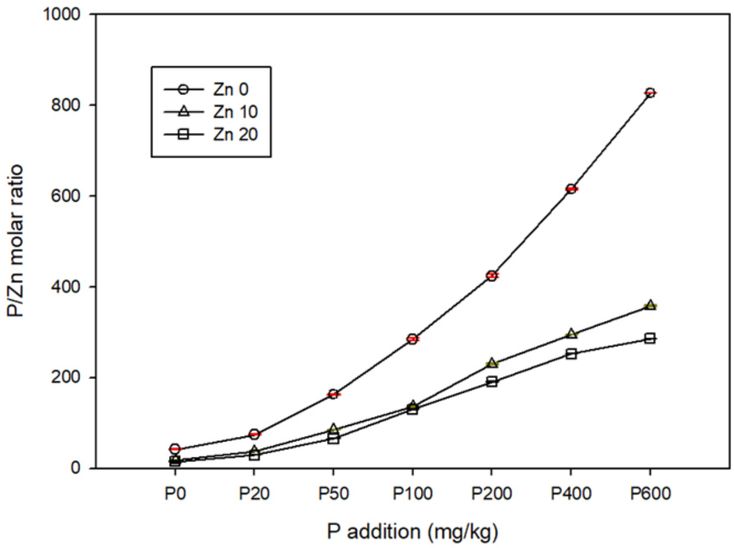

Fig. 4. Effects of different levels of P and Zn on P-Zn Molar Ratios in Greengram Shoots

Table 3. Correlation of different parameters under combined addition of P and Zn in greengram

	P level	Zn level	P conc.	Zn conc.	P/Zn	Soil pH	Rhizosphere pH
P level	1						
Zn level	.000	1					
P conc.	.901**	-.002	1				
Zn conc.	-.479**	.808**	-.517**	1			
P/Zn	.784**	-.419**	.781**	-.754**	1		
Soil pH	.901**	.010	.951**	-.514**	.760**	1	
Rhizosphe-ric pH	.876**	.121	.928**	-.424**	.716**	.934**	1

*** Correlation is significant at the 0.01 level (2-tailed)*

On average, considering the three Zn treatments, the soil pH increased from 4.22 to 4.92, and the rhizospheric extract pH increased from 4.89 to 6.27 as the P levels increased from 0 to 600 mg kg^{-1}. In greengram shoots, the P concentration and P/Zn molar ratio were positively correlated with both pH values. However, the Zn concentration in the shoots showed an inverse relationship with both pH values.

These findings suggest that the addition of P and Zn can influence soil and rhizospheric pH, with higher P levels generally resulting in increased pH. The pH values in the rhizosphere can have implications for nutrient availability and uptake by plants, as well as for the relationships between P, Zn, and other nutrients in the soil-plant system.

4. DISCUSSION

Our findings indicate that applying P and Zn in appropriate proportions enhances the performance of greengram plants compared to individual treatments of P or Zn. We found that the absorption of P by greengram shoots increased significantly when both P and Zn were added up to a certain limit. This finding aligns with previous research conducted on maize [21]. However, applying high doses of P (>400 mg/kg soil) resulted in P toxicity in the lower leaves, as the shoot P concentration exceeded the phytotoxic limit of 2% [22]. Additionally, the severity of Zn insufficiency was exacerbated, irrespective of Zn addition. The increase in P content was associated with a reduction in Zn concentrations in the shoots, indicating a possible competition between the two nutrients. Phosphate application resulted in a significant reduction in grain zinc (Zn) concentration in wheat by 16.6%, maize by 20.2%, but had no significant impact on rice; although soil Zn concentration remained unaffected, it notably decreased root Zn concentration by 9.94% across various crops due to reduced colonization of roots by AMF, emphasizing the influence of rhizosphere processes [23]. Increased P fertilization rapidly improved crop biomass in conditions of low-available P soil, and the dilution effect may play a key role in reducing Zn concentrations, especially when the amount of P added increases from insufficient to adequate levels [24]. Similar results have been reported for various cereals and pulses, such as rice [7,25]; wheat [22,26]; maize [27]; cowpea [28]; bean [29] and chia [30].

Interestingly, when the P rate was increased from optimal to excessive levels, there was no improvement in crop yield, but the Zn content steadily declined. This decline cannot be fully explained by the dilution effect. The reduced Zn concentration in the plant due to higher levels of P addition may be attributed to changes in Zn availability in the soil rhizosphere [31-33]. Surprisingly, excessive inorganic P addition raised the pH of both the soil and the rhizosphere. This is because roots have a tendency to increase pH rather than decrease it [34,35]. The pH of the soil and rhizosphere of greengram plants grown in red and lateritic soils increased as a result of the plants' release of bicarbonate (HCO^{3-}) or hydroxyl ions (OH^{-}) to maintain electrical neutrality across the soil-root interface when higher P is applied to low P soil [36]. Similar outcomes have been observed with higher NO^{3-} absorption [37,38]. The decrease in Zn uptake by greengram shoots may be attributed to the increase in soil and rhizospheric pH, as it can immobilize Zn [15,39] and lead to an elevated P-Zn molar ratio above the optimal level. This may explain the insufficient Zn absorption at higher P addition in P-deficient soil. Therefore, it is recommended to fertilize P-deficient soils with the appropriate amounts of P and Zn, below which the root and rhizosphere qualities of the plants begin to suffer [18].

5. CONCLUSION

Our study highlights the importance of applying P and Zn in appropriate proportions to enhance the performance of greengram plants. The absorption of P by greengram shoots increased significantly when both P and Zn were added within a certain limit. However, excessive P application led to P toxicity and decreased Zn concentrations in the shoots. We observed a potential competition between P and Zn, with higher P levels reducing Zn uptake. Additionally, excessive inorganic P application resulted in increased soil and rhizospheric pH, which may immobilize Zn and further affect its uptake. Our findings emphasize the need for balanced P and Zn fertilization in P-deficient soils to optimize root and rhizosphere qualities of greengram plants.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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