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# PHOSPHORUS AND ZINC SYNERGIES WITH ZEOLITE, LATERITE, AND BIOCHAR FOR MITIGATING CADMIUM TOXICITY IN MAIZE

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**Abstract**

Toxicity induced by cadmium (Cd) heavy metal is an acute and potential threat to living organisms. It can cause cancer in humans while adversely affecting the growth of plants. The use of phosphorus (P) and zinc (Zn) is critical to resolve this critical issue. Homeostasis of these two nutrients in plants can potentially minimize the toxic effects of Cd. Furthermore, biochar, zeolite, and laterite are also becoming popular. These amendments also effectively minimize Cd toxicity due to their high absorption capacity. That's why the current study was planned to check the effectiveness of P and Zn combination with biochar, zeolite, and laterite in alleviating Cd stress in maize. A total of 16 treatments (No P and Zn, P, Zn, P+Zn applied under control, zeolite, laterite, and biochar) were applied in 3 replications following a completely randomized design. Results showed that application of Zn+P caused significant enhancement in plant height (30.17%), chlorophyll contents (26.56%), root fresh weight (84.13%), shoot dry weight (91.89%), shoot Cd concentration (47.33%) and root Cd concentration (65.43%) compared to no P and Zn under zeolite. In conclusion, P+Zn application under zeolite can potentially mitigate the Cd toxicity in maize. More investigations are suggested at the field level under variable agro climate zones to declare P+Zn under zeolite as the best amendment for alleviating Cd stress in maize.

**Key words:** Biochar; Cadmium; Chlorophyll contents; Growth attributes; Laterite; Nutrients; Zeolite

## Introduction

Cadmium (Cd) induced toxicity in maize plants is a serious threat. It minimizes growth by impairing photosynthesis (Rehman *et al.,* 2023; Naseem *et al.,* 2023). This decrease in photosynthesis is associated with disrupting and inhibiting photosynthetic enzyme-related proteins and degradation of chlorophyll contents by Cd (Younis *et al.,* 2014; Chen *et al.,* 2022; Shahzad *et al.,* 2024; Dawar *et al.,* 2023). Plants under Cd stress lose their membrane integrity due to oxidative stress, which alters the enzyme activities (Dawar *et al.,* 2023; Shahzad *et al.,* 2024; Sana *et al.,* 2024). It is also associated with antagonistic relationships with other essential nutrients, i.e., zinc. Higher uptake if Cd decreases the concentration of iron (Fe) and Zn in plants, which creates nutrient imbalance and decreases plant growth (Li *et al.,* 2020).

On the other hand, researchers have reported the positive role of phosphorus (P) and Zn in alleviating cadmium (Cd) stress in plants. Phosphorus application minimizes the Cd uptake and translocation, which causes improvement in antioxidant-based defense mechanisms and photosynthetic activity (Zhang *et al.,* 2024; Jia *et al.,* 2024). It is also associated with better uptake of essential nutrients, i.e., K, Zn, and Mn, which are key in root growth promotion (Zhang *et al.,* 2024). The optimum presence of Zn decreased Cd absorption, improving chlorophyll biosynthesis (Hassan *et al.,* 2022; Umair *et al.,* 2024). It also stabilizes the membrane and boosts the activities of antioxidants such as peroxidase, superoxide dismutase, and catalase. Improving the activities of antioxidants is a key factor for minimizing oxidative stress (Arshad *et al.,* 2016).

In addition to the above, zeolite, laterite, and biochar have also been reported as promising in minimizing plant Cd toxicity. Biochar addition as amendment decreases Cd mobilization and plant uptake (Feng *et al.,* 2022; Jiang *et al.,* 2022). Furthermore, mixing it with nano-zeolite or zinc can enhance plant growth and decrease Cd uptake (Farooq *et al.,* 2020; Feng *et al.,* 2022). The effectiveness of zeolite in immobilization of Cd is associated with an increase in soil pH and its ion exchange capacity (Guo *et al.,* 2021; Hannan *et al.,* 2024). Similarly, using laterite as a soil amendment improves the availability of nutrients to plants due to its acidic pH. It also improves the soil's physical conditions, which facilitates root growth. Improvement in root growth increases nutrients and water uptake, providing a favorable environment for better plant growth (Lamidi *et al.,* 2018).

That is why the current study was planned to examine the effect of P and Zn combinations with and without zeolite, laterite, and biochar on maize growth under Cd stress. The study covers the knowledge gap regarding using P and Zn under zeolite, laterite, and biochar to induce tolerance in maize against Cd toxicity. The study aimed to select the best combination of P and Zn with or without zeolite, laterite, and biochar to improve maize growth under Cd toxicity. It is hypothesized that the combined application of P and Zn might effectively enhance maize growth under Cd stress when applied with zeolite, laterite, and biochar.

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## Material and Methods

**Experimental setup:** A pot experiment was conducted in the greenhouse of Muhammad Nawaz Shareef (MNS) University of Agriculture, Multan Pakistan (C-Block, 30.14086, 71.443518) to assess the efficacy of mineral nutrients (P and Zn) and soil amendments (zeolite, laterite, and biochar) against Cd toxicity in maize crop.

**Treatment plan and experimental design:** The treatments include P application at 140 kg $ha^{-1}$ (70 mg $kg^{-1}$ soil) while Zn 3 kg $ha^{-1}$ (1.5 mg $kg^{-1}$ soil). Both fertilizers were applied as soil applications, considering the pre-experimental soil characteristics. Zeolite, laterite, and biochar were applied at 10 tons $ha^{-1}$ (5g $kg^{-1}$ soil) in pre-spiked soil with a Cd concentration of 5 mg $kg^{-1}$. The soil spiking was done using analytical grade Cd $(NO_3)_2$ salt. After spiking, the soil was incubated for 30 days at field capacity (70%) moisture in large-sized polythene bags in a greenhouse at 28 °C. A total of 16 treatments were applied in 3 replications following a completely randomized design (CRD).

**Pre-experimental soil characterization:** This study collected surface soil from the MNS-University of Agriculture Multan research area, dried and sieved from a 2 mm sieve, and homogenized (Petersen *et al.,* 1986). After that, it was characterized by following standard protocols in the laboratory. The references to methods used to analyze soil characteristics and values are provided in Table 1.

**Table 1. Physico-chemical properties of soil used in the experiment.**

| Soil properties | Results | References |
|---|---|---|
| EC (dS $m^{-1}$) | 0.35 | (Rhoades *et al.,* 1996) |
| pHs | 7.40 | (McLean *et al.,* 1982) |
| Soil organic matter (%) | 0.38 | (Nelson & Summer, 1982) |
| Av. P (mg $kg^{-1}$) | 6.70 | (Kuo *et al.,* 2018) |
| Av. K (mg $kg^{-1}$) | 135 | (Pratt and Norman, 2016) |
| AB-DTPA Zn (mg $kg^{-1}$) | 0.28 | (Hanlon & Kalra, 1998) |
| AB-DTPA Cd (mg $kg^{-1}$) | 0.13 | (Hanlon & Kalra, 1998) |
| Texture | Loam | (Gee & Bauder, 1986) |

Av. = Available; Cd = Cadmium; Zn = Zinc; EC = Electrical conductivity
AB-DTPA = Ammonium bicarbonate-diethylenetriaminepentaacetic acid

**Seeds collection and sowing:** The seeds of maize variety YH-5427 were purchased from certified seed dealer. After removal of dead and broken seeds manually, 5 seeds were sown in each pot (15 cm width × 35 cm depth). Once seeds were germinated, 3 seedlings were maintained in each pot. Throughout the experiment, the pots were irrigated at the rate of 70% field capacity.

**Harvesting and data collection:** After 60 days of sowing, the plants were harvested for data collection. The fresh weight of samples was noted using top weight balance. For dry weight, samples were oven-dried at 65˚C for 72h. The dried weight was taken on an analytical balance. A measuring tape was used to measure height. However, SPAD (Chlorophyll Meter SPAD-502 Plus) was used for chlorophyll contents.

**Nutrients and cadmium:** Contents of P, Zn, and Cd were measured in homogenized samples of maize plants following the standard protocols and procedures. For this purpose, plant samples were oven dried, grinded, and sieved with a 1 mm sieve. One gram of plant material (shoot and root) was digested using di-acid digestion ($HNO_3$:$HClO_4$ 2:1 ratio) (Miller, 1998). The P was determined using a Hitachi UH5300 spectrophotometer following the method described by (Estenfan *et al.,* 2013), while Zn and Cd contents were analyzed on an atomic absorption spectrophotometer (Analytik Jena nova P400) (Hanlon *et al.,* 1998).

**Statistical analysis**

Standard statistical analysis (Steel *et al.,* 1997) was performed using Origin Pro 2021 software. The paired comparison was applied for the determination of treatment significance at $p \leq 0.05$ by applying Fisher's LSD. A hierarchical cluster plot was made to determine the most representative attribute. A Convex hull cluster plot was also made to determine the best treatment using Origin Pro 2021 software (Anon., 2021).

## Results

**Plant height and chlorophyll:** Results showed that application of P+Zn caused significant increases of 29.62%, 30.17%, 30.69%, and 27.76% in plant height of maize compared to no P and Zn when applied under control, zeolite, laterite, and biochar, respectively. Applying Zn as treatment resulted in 22.97% and 18.79% significant, while 11.27% 12.03%, and non-significant enhancement in maize plant height over no P and Zn at control, biochar, zeolite and laterite, respectively. In the case of P at control and biochar, a significant improvement, i.e., 17.87% and 19.53%, while at zeolite and laterite, a non-significant increase (5.97% and 7.70%) was observed in plant height from no P and Zn respectively. On an average, zeolite with P+Zn showed the highest percentage increase (9.54%) in plant height of maize compared to control with P+Zn (Fig. 1A).

Application of P+Zn performed significantly best and caused improvement of 27.29%, 26.56%, 18.59% and 17.17% in chlorophyll contents of maize over no P and Zn under control, at zeolite, laterite and biochar respectively. Sole application of Zn caused 11.81% significant increase at control, while 0.20% 5.05%, and 6.02% non-significant increase in maize chlorophyll contents over no P and Zn zeolite, laterite and biochar respectively. Treatment P at control, laterite and biochar resulted in a non-significant increase, i.e., of 5.64%, 6.25% and 23.62%, while a decrease at zeolite (2.73%) in chlorophyll contents compared to no P and Zn, respectively. On an average, biochar with no P+Zn showed the highest percentage increase (20.07%) in chlorophyll contents of maize compared to control with no P+Zn (Fig. 1B).

**Fresh and dry weight (shoot and root):** It was observed that treatment showed P+Zn significantly best results regarding enhancement, i.e., 174.62%, 236.06%, 71.87%, and 52.35% in fresh shoot weight of maize compared to no P and Zn under control, at zeolite, laterite, and biochar respectively. Treatment Zn resulted in 26.90%, 86.01%, and 10.18% significant increases at control, zeolite, and laterite, respectively, while 5.18% significant decrease at biochar in shoot fresh weight over no P and Zn. Applying sole P at control, zeolite, laterite, and biochar significantly increased, i.e., 113.20%, 150.00%, 44.79%, and 41.69% in fresh shoot weight compared to no P and Zn, respectively. On an average, sole biochar addition showed the highest percentage increase (93.46%) in fresh shoot weight of maize compared to no P+Zn + control (Fig. 2A).

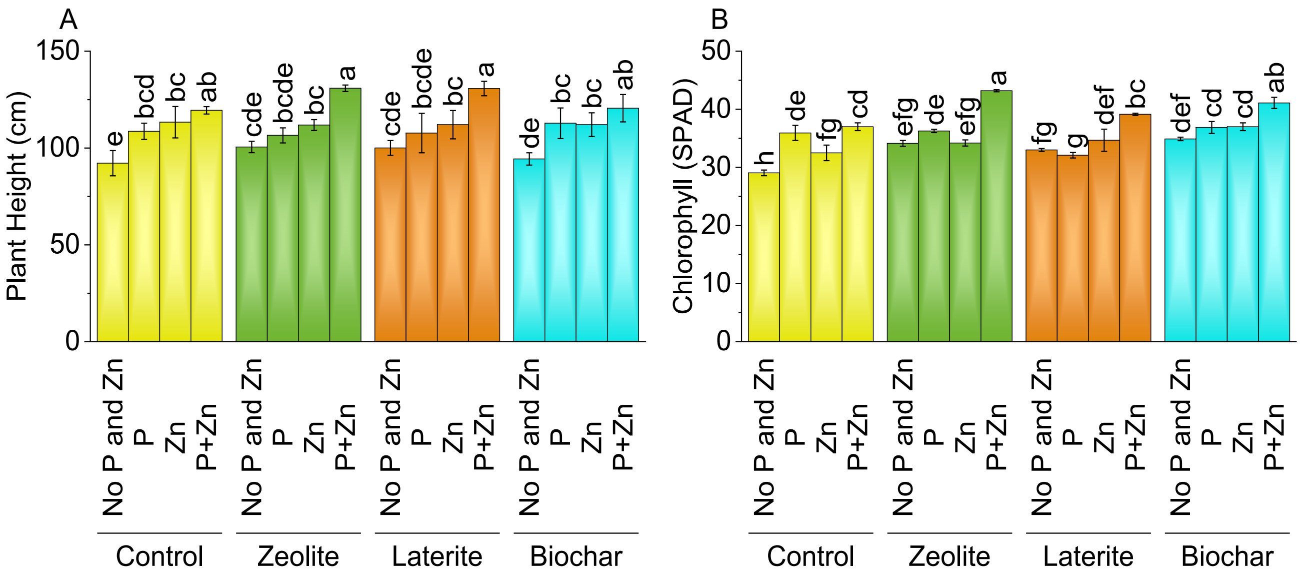


Fig. 1. Effect of sole and combined application of phosphorus (P) and zinc (Zn) with and without zeolite, laterite and biochar on plant height (A) and chlorophyll contents (B) of maize. Bars are showing mean of 3 replicates ± SE. Different letters on bars showed significant changes compared by applying Fisher's LSD ($p \leq 0.05$).

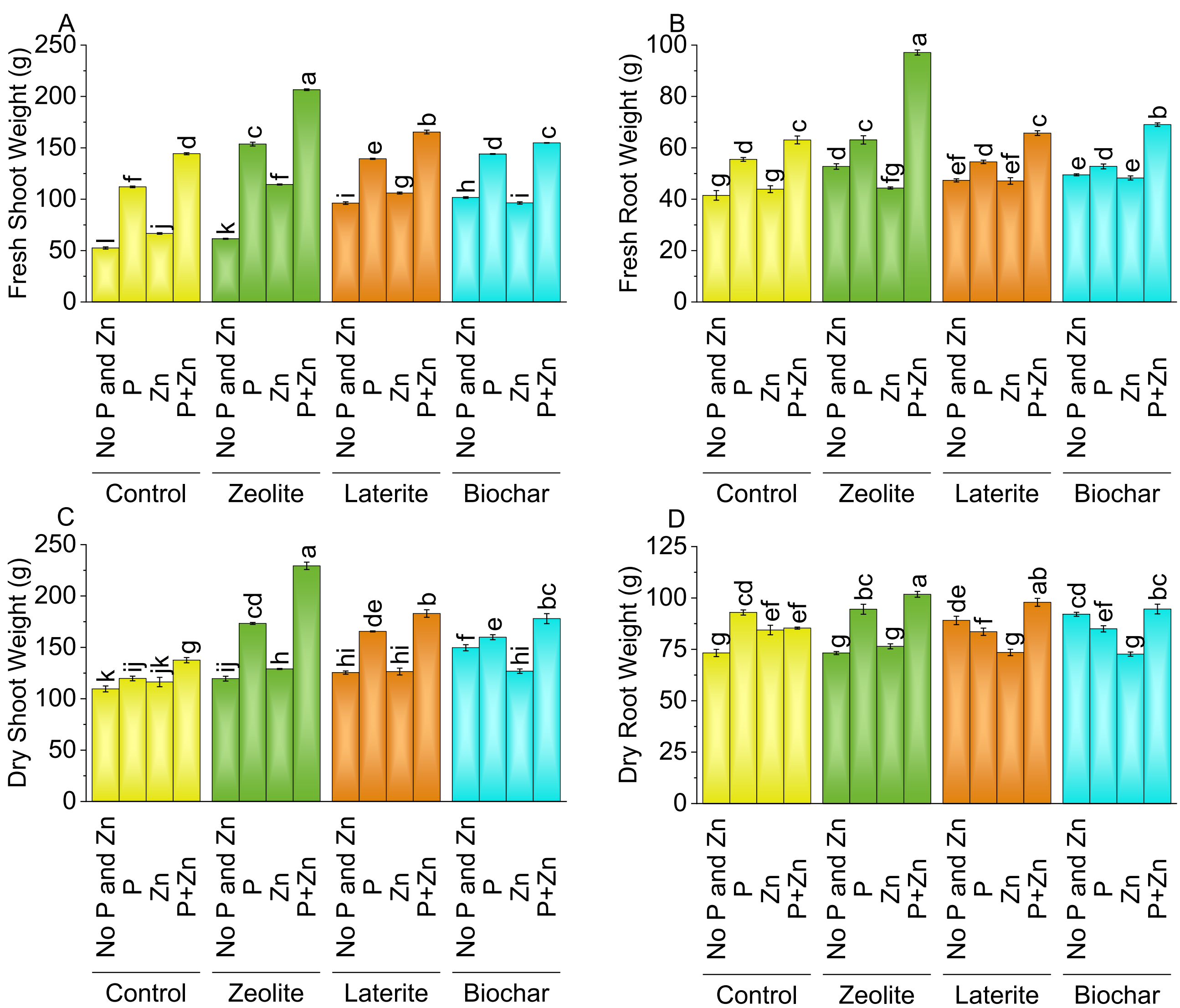


Fig. 2. Effect of sole and combined application of phosphorus (P) and zinc (Zn) with and without zeolite, laterite and biochar on fresh shoot weight (A), fresh root weight (B), dry shoot weight (C) and dry root weight (D) of maize. Bars are showing mean of 3 replicates ± SE. Different letters on bars showed significant changes compared by applying Fisher's LSD ($p \leq 0.05$).

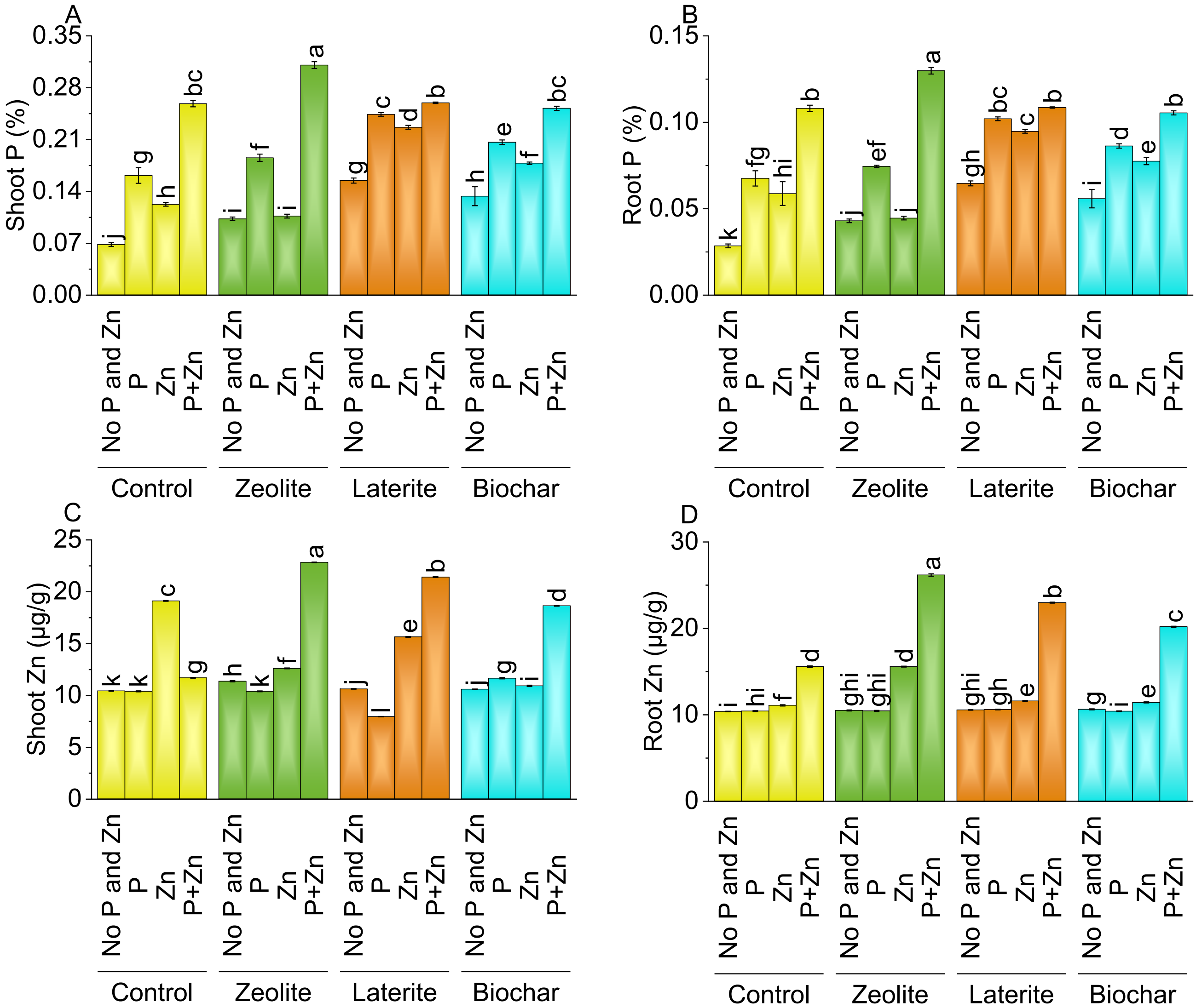


Fig. 3. Effect of sole and combined application of phosphorus (P) and zinc (Zn) with and without zeolite, laterite and biochar on shoot P (A), root P (B), shoot Zn (C) and root Zn (D) of maize. Bars are showing a mean of 3 replicates ± SE. Different letters on bars showed significant changes compared by applying Fisher's LSD (p≤0.05).

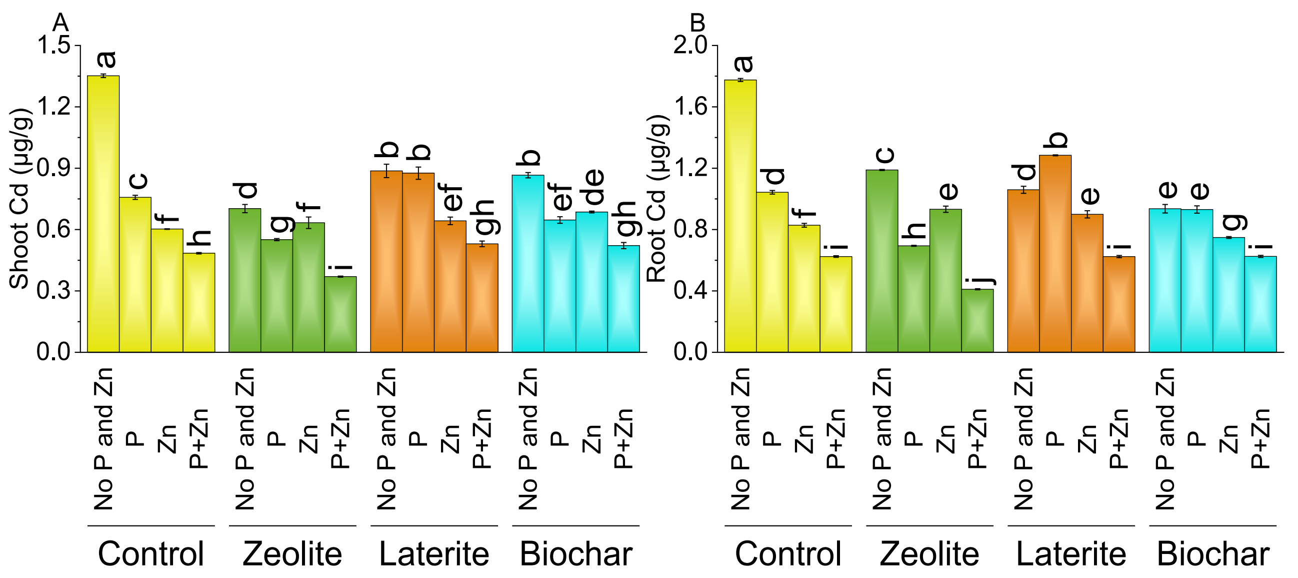


Fig. 4. Effect of sole and combined application of phosphorus (P) and zinc (Zn) with and without zeolite, laterite, and biochar on shoot Cd (A) and root Cd (B) of maize. Bars are showing a mean of 3 replicates ± SE. Different letters on bars showed significant changes compared by applying Fisher's LSD (p≤0.05).

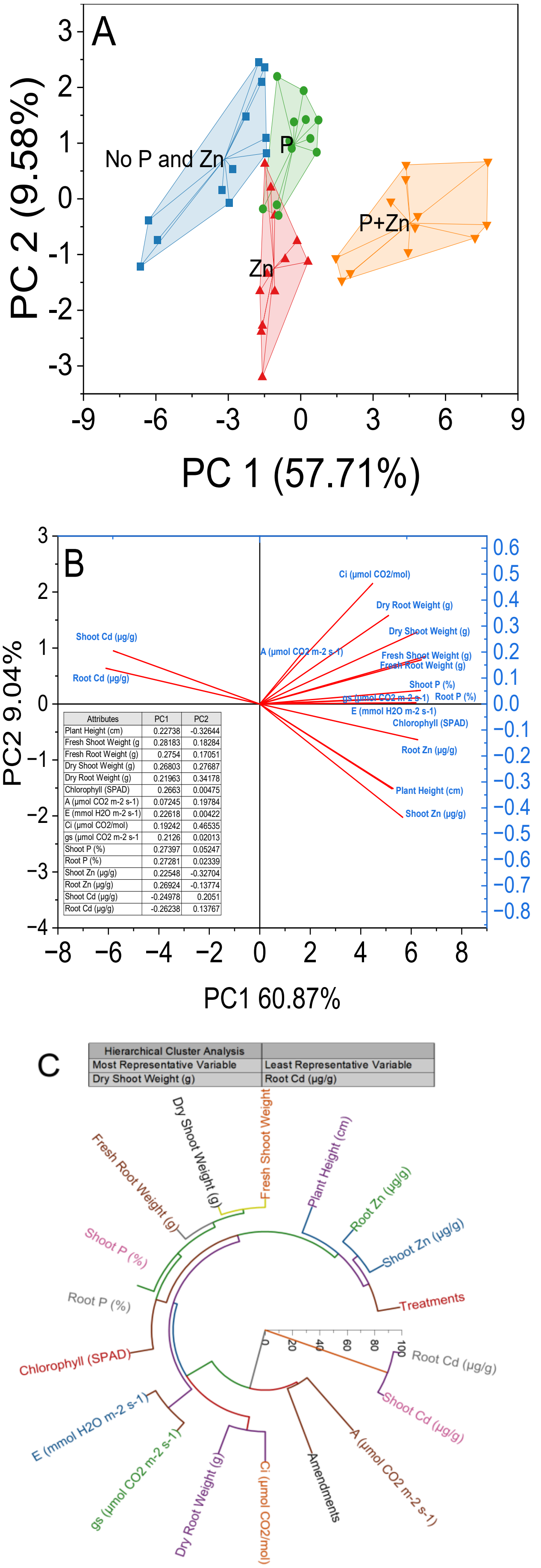


Fig. 5. Convex hull for P and Zn combination application (A), attributes (B), and hierarchical cluster plot (C) for studied attributes.

Resulted showed that the application of P+Zn caused a significant increase, i.e., 51.97%, 84.13%, 38.87%, and 39.46% in fresh root weight of maize compared to no P and Zn under control, zeolite, laterite, and biochar respectively. Treatment Zn resulted in 5.78% non-significant increase at control in root fresh weight over no P and Zn. However, zeolite showed significant while laterite and biochar showed a non-significant decrease of 15.87, 0.49% and 2.49%, respectively, in root fresh weight over no P and Zn. Treatment P at control, zeolite, laterite and biochar significantly enhanced 33.73%, 19.66%, 15.21%, and 6.60% in fresh root weight over no P and Zn, respectively. On an average, zeolite addition with P+Zn caused the highest percentage improvement (53.96%) in fresh root weight of maize compared to P+Zn + control (Fig. 2B).

A significant improvement, i.e., 25.54%, 91.89%, 45.78%, and 18.93% in dry shoot weight, was noted where P+Zn was applied as treatment compared to no P and Zn under control, zeolite, laterite and biochar respectively. Sole application of Zn with zeolite showed a significant increase (7.91%), while biochar showed a significant decrease (15.24%) in dry shoot weight over no P and Zn with zeolite and biochar, respectively. In the case of control and laterite, the application of Zn showed a non-significant increase of 6.15% and 0.78% in dry shoot weight compared to no P and Zn with control and laterite, respectively. A significant enhancement was observed in dry shoot weight of maize when P was applied with control (9.28%), zeolite (44.99%), laterite (31.93%), and biochar (6.85%) compared to no P and Zn respectively. On an average, zeolite application with P+Zn resulted in the highest percentage increase (66.75%) in dry shoot weight of maize over P+Zn + control (Fig. 2C).

A significant enhancement, i.e., 39.03% and 9.88%, while a non-significant increase of 2.75% in dry root weight was observed where P+Zn was added as treatment over no P and Zn at zeolite, laterite, and biochar, respectively. However, treatment P showed a significant increase in dry root weight in control compared to no P and Zn + control. Sole application of Zn + control showed a significant increase (15.25%) in dry root weight compared to no P and Zn + control, while zeolite showed non-significant enhancement (4.46%) in dry root weight over no P and Zn + zeolite. However, laterite and biochar caused a significant decline (17.48% and 21.07%) in dry root weight over no P and Zn with laterite and biochar, respectively. Application of sole P at control (26.90%) and zeolite (29.10%) gave a significant increase in dry root weight. However, laterite (6.18%) and biochar (7.68%) showed a significant decrease in dry root weight compared to respective no P and Zn. On an average, biochar application with no P and Zn resulted in the highest percentage increase (66.75%) in dry root weight of maize over no P and Zn + control (Fig. 2D).

**Shoot and root (P and Zn concentration):** Treatment P+Zn significantly best results regarding enhancement, i.e., 278.69%, 201.71%, 67.85%, and 88.85% in shoot P of maize over no P and Zn under control, at zeolite, laterite, and biochar, respectively. Applying Zn caused 79.63%, 46.49%, and 33.38% significant increases at control, laterite, and biochar, respectively, while 3.63% non-significant increase at zeolite in shoot P over no P and Zn. In the case of sole P addition at control, zeolite, laterite, and biochar resulted in significant increases, i.e., 136.71%, 80.19%, 57.78%, and 54.56% in shoot P over no P and Zn, respectively. On an average, sole laterite addition showed the highest percentage increase (126.60%) in shoot P of maize than no P+Zn + control (Fig. 3A).

It was noted that P+Zn resulted in a significant increase, i.e., 279.23%, 201.93%, 67.90% and 88.98% in root P of maize than no P and Zn under control, zeolite, laterite and biochar respectively. Sole Zn addition caused 106.14%, 46.56%, and 38.89% significant increases at control, laterite, and biochar in root P compared to no P and Zn. However, at zeolite, applying Zn caused non-significant increase of 3.67% in root P over no P and Zn. Treatment P at control, zeolite, laterite and biochar significantly enhanced 137.02%, 73.21%, 57.82%, and 54.66% in root P compared to no P and Zn, respectively. On an average, sole laterite addition showed the highest percentage increase (126.84%) in root P of maize than no P+Zn + control (Fig. 3B).

A significant enhancement, i.e., 12.09%, 100.84%, 101.47%, and 75.90%, was observed in shoot Zn at control, zeolite, laterite, and biochar, respectively, where P+Zn was applied compared to no P and Zn. On the other hand, treatment Zn resulted in significant enhancement of 83.15%, 10.91%, 47.19%, and 3.09% in shoot Zn than no P and Zn at control, zeolite, laterite, and biochar, respectively. Treatment P showed a significant decrease (8.58%) in shoot Zn compared to no P and Zn at zeolite. On an average, zeolite application with P+Zn resulted in the highest percentage increase (95.17%) in shoot Zn of maize over P+Zn+control (Fig. 3C).

Results showed that P+Zn caused 49.98%, 149.07%, 117.46%, and 89.91% enhancement in root Zn over no P and Zn under control, zeolite, laterite, and biochar, respectively. Treatment Zn resulted in a significant increase (6.81%, 48.21%, 9.83 and 7.58%) in root Zn compared to no P and Zn at control, zeolite, laterite, and biochar, respectively. Non-significant enhancement, i.e., 0.51% and 0.42%, was observed in root Zn of maize when P was applied at control and laterite over no P and Zn, respectively. On an average, zeolite application with P+Zn resulted in the highest percentage increase (68.00%) in root Zn of maize over P+Zn + control (Fig. 3D).

**Shoot and root Cd concentration:** In the case of P+Zn, a significant decline, i.e., 64.18%, 47.33%, 40.23%, and 39.75% in shoot Cd of maize over no P and Zn under control, zeolite, laterite, and biochar, respectively. Sole Zn addition caused 55.43%, 9.85%, 27.54%, and 20.79% significant decrease at control, laterite, and biochar in shoot Cd compared to no P and Zn. Treatment P at control, zeolite, and biochar resulted in a significant decline of 43.96%, 21.59%, and 25.31% in shoot Cd over no P and Zn, respectively. On an average, zeolite + no P+ no Zn addition showed the highest percentage decrease (48.03%) in shoot Cd of maize over no P+ no Zn + control (Fig. 4A).

A significant decrease, i.e., 64.84%, 65.43%, 41.07%, and 33.21%, was observed in root Cd at control, zeolite, laterite, and biochar, respectively, where P+Zn was applied compared to no P and Zn. On the other hand, treatment Zn resulted in a significant decline of 53.33%, 21.53%, 15.11% and 20.04% in root Cd than no P and Zn at control, zeolite, laterite, and biochar, respectively. Treatment P showed a significant decrease (41.22% and 41.58%) at control and zeolite and a significant increase (21.24%) at laterite in root Cd over no P and Zn. On an average, biochar application with no P+ no Zn resulted in the highest percentage decrease (47.28%) in root Cd of maize over P+Zn+control (Fig. 4B).

**Principal component analysis (PCA):** The principal component analysis (PCA) results indicate that the first principal component (PC 1) explains 57.71% of the variance, while the second principal component (PC 2) accounts for 9.58%, together explaining 67.29% of the variance in the data. The treatment combinations show distinct patterns along these components. The No P and Zn treatment generally has lower negative scores on PC 1, indicating its separation from other treatments. The P+Zn treatment, in contrast, exhibits high positive scores on both PC 1 and PC 2, particularly in instances where PC 1 scores exceed 4, demonstrating its distinctiveness. The P treatment displays moderate to low scores on PC 1, with scores on PC 2 varying from positive to negative, while the Zn treatment shows a wider range of scores along both components, suggesting greater variation. Overall, the P+Zn combination shows the most distinct profile, while the No P and Zn treatment concentrates in the negative region of PC 1. The P and Zn treatments show more variability, particularly along PC 2, highlighting that phosphorus and zinc individually influence the system differently (Fig 4A).

For PC1, fresh shoot weight (g) (0.28183), fresh root weight (g) (0.2754), and dry shoot weight (g) (0.26803) have the highest positive loadings, indicating that these attributes have a strong influence on the variance along PC1. Similarly, root P (%) (0.27281) and shoot P (%) (0.27397) also show positive loadings on PC1, reflecting their contribution to the overall variance in plant growth and nutrient composition. On the other hand, shoot Cd (μg/g) (-0.24978) and root Cd (μg/g) (-0.26238) show negative loadings on PC1, suggesting an inverse relationship with other growth-related attributes. For PC2, dry root weight (g) (0.34178) and root P (%) (0.02339) have higher positive contributions, while shoot Zn (μg/g) (-0.32704) and plant height (cm) (-0.32644) show moderate negative loadings, indicating that these attributes are more associated with the second principal component. Ci (μmol $CO_2$/mol) (0.46535) shows the strongest positive correlation with PC2, reflecting its significant impact on the variance related to photosynthetic efficiency and gas exchange parameters (Fig. 5B).

Shoot P (%) and root P (%), with identical values of 0.48883, were strongly related, indicating similar phosphorus content in both plant parts. Similarly, shoot Cd (μg/g) and root Cd (μg/g), both with values of 4.73902, clustered together, suggesting a close relationship in cadmium accumulation in the shoot and root. Fresh shoot weight (g) and dry shoot weight (g), with values of 6.09029, were also grouped closely, reflecting their shared contribution to plant biomass. The attributes E (mmol $H_2O$ $m^{-2}$ $s^{-1}$) and gs (µmol $CO_2$ $m^{-2}$ $s^{-1}$) clustered together, with values of 7.39663, indicating their strong correlation in water and gas exchange processes. Fresh root weight (g), with a value of 9.17872, showed a weaker association with shoot Zn (µg/g) and root Zn (µg/g), both valued at 11.62642, suggesting that zinc uptake in both the shoot and root is somewhat related to root biomass. On the other hand, chlorophyll (SPAD) (16.71364) and plant height (cm) (20.67115) were more distantly related to other attributes, reflecting their distinct roles in plant health and growth. The attributes dry root weight (g) (22.23498) and Ci (µmol CO2/mol) (22.23498) formed a separate cluster, indicating a distinct relationship between root biomass and intercellular $CO_2$ concentration, which is influenced by photosynthetic activity (Fig. 5C).

**Pearson correlation:** Plant height showed positive correlations with fresh shoot weight (0.61), fresh root weight (0.58), dry shoot weight (0.51), and chlorophyll content (SPAD) (0.59), indicating that taller plants tend to have higher biomass and chlorophyll levels. It also exhibited moderate positive correlations with shoot phosphorus (P) (0.63), root zinc (Zn) (0.67), and shoot cadmium (Cd) (0.67). Fresh shoot weight was strongly positively correlated with fresh root weight (0.84), dry shoot weight (0.91), dry root weight (0.71), and chlorophyll content (0.77), suggesting a close relationship between shoot growth, root development, and chlorophyll production. Similarly, fresh shoot weight had high correlations with shoot P (0.87) and root P (0.85), alongside moderate correlations with root Zn (0.72) and shoot Zn (0.45).

Fresh root weight also displayed strong correlations with dry shoot weight (0.87), dry root weight (0.70), and chlorophyll (0.78). It showed a particularly strong relationship with root Zn (0.80) and moderate correlations with shoot Cd (0.65). Dry shoot weight was positively associated with dry root weight (0.72), chlorophyll (0.73), and shoot P (0.77) and demonstrated positive correlations with root Zn (0.74), root Cd (0.65), and shoot Cd (0.55). Dry root weight followed a similar trend, showing positive relationships with chlorophyll (0.56), root Zn (0.66), and shoot P (0.70), with moderate correlations with shoot Cd (0.61) and root Cd (0.56).

Chlorophyll content (SPAD) exhibited positive correlations with shoot P (0.55), root P (0.48), and shoot Zn (0.59) and moderate correlations with shoot Cd (0.44) and root Zn (0.55). The strong correlation between shoot P and root P (0.99) highlights their mutual influence on plant nutrient dynamics. Additionally, shoot P and root P were positively associated with shoot Zn (0.51) and root Zn (0.66). Root Zn showed strong correlations with root Cd (0.81) and shoot Cd (0.68), while shoot Zn demonstrated moderate positive relationships with shoot Cd (0.59) and root Cd (0.61) (Fig. 6).

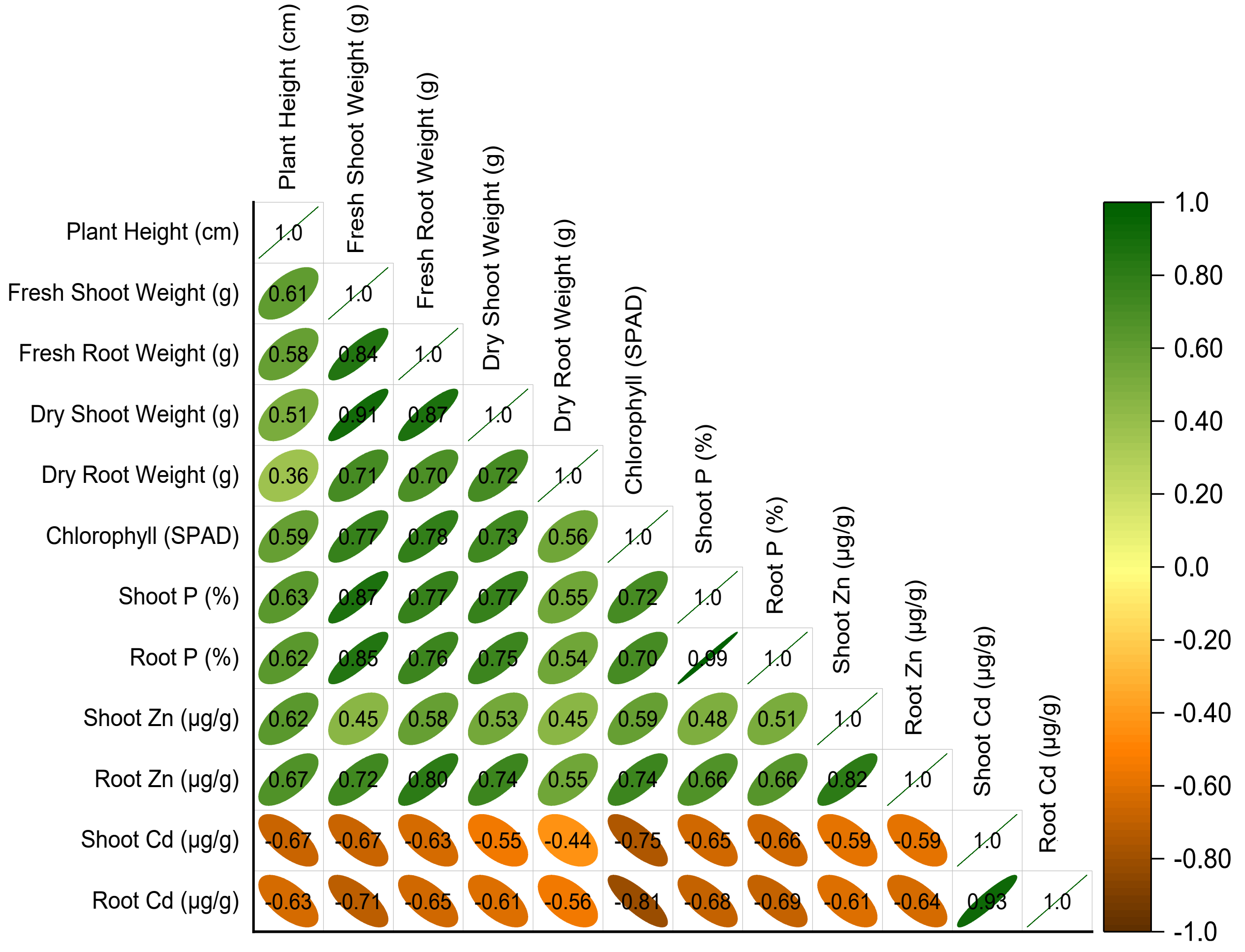

Fig. 6. Pearson correlation for studied attributes.

## Discussion

**Cd immobilization:** In the current study, it is evident that P and Zn combined application under zeolite performance was best for alleviating Cd toxicity and growth attributes of maize. Zeolite possesses a high surface area and cation exchange capacity, which caused the immobilization of Cd ions in soil (Moeen *et al.,* 2020; Ma *et al.,* 2022). The low availability of Cd in the soil allows plants to improve root growth compared to control treatment plants, which usually suffer from Cd toxicity (Riaz *et al.,* 2020). In addition, P application also decreases Cd mobilization in soil due to the formation of the P-Cd complex (Lee & Hong, 2016). Similar ionic radii of Cd and Zn create competition for their uptake in plants. When Zn is in ample concentration in soil, the Cd movement inside plants' roots decreases due to this competition (Wang *et al.,* 2016). Thus, both P and Zn are essential nutrients that improve root growth. Similarly, results were also noted in the current study, where root dry weight improvement was the most representable attribute of maize, and P and Zn were applied under zeolite in Cd toxicity.

**Chlorophyll content and photosynthetic efficiency:** Under Cd stress, plants usually degrade chlorophyll. It also impairs the chlorophyll biosynthesis by inducing the oxidative stress in plants (Zhang *et al.,* 2020). Adding Zn as an amendment is pivotal in decreasing such adverse effects. It protects the photosynthetic machinery and stabilizes the chlorophyll structure. Furthermore, Zn also acts as a cofactor in enzymes of chlorophyll synthesis and scavenging of reactive oxygen species (ROS), which facilitate the improvement of photosynthetic pigments (Amiri *et al.,* 2016; Hassan *et al.,* 2022; Ganguly *et al.,* 2022). Applying P supports ATP synthesis and RuBP regeneration in the Calvin cycle, thus boosting photosynthesis efficiency (Campbell & Sage, 2006).

**Gas exchange attributes:** Activating carbonic anhydrase enzyme by applying Zn as treatment facilitates stomata opening and closing for $CO_2$ fixation (Escudero-Almanza *et al.,* 2012). Better uptake of P increases root growth, which assists in optimum water and nutrients uptakes. These nutrients strengthen the plants to withstand stress conditions while water uptake regulates the transpiration rate in plants (Wittenmayer & Merbach, 2005; Aroca *et al.,* 2012).

**Growth attributes:** Zeolite provides a friendly environment by improving soil water retention, aeration, and nutrient bioavailability. Better uptake of P and Zn facilitates cell elongation and division, which are critical for root and shoot growth (Xue *et al.,* 2016). In plants, Zn also assists in activating auxin, while P provides energy essential for plants' growth (Begum *et al.,* 2016). Our findings also align with the above arguments where P and Zn application under zeolite caused significant enhancement in root and shoot fresh as we as dry weights under Cd toxicity.

## Conclusion

It is concluded that the combined application of P and Zn with zeolite can potentially improve the growth of maize plants under Cd toxicity. Application of P+Zn with zeolite can improve the dry weight of the shoot, which is the most representative attribute for the enhancement of maize growth under Cd stress. This enhancement in shoot dry weight was associated with better P and Zn uptake and less Cd uptake. Such Zn and P concentration improvements facilitate the development of chlorophyll contents, which was linked with improved photosynthetic rate, transpiration rate, and stomatal conductance. Growers of maize are recommended to apply Zn+P along with zeolite to achieve better maize growth under Cd toxicity. From a future perspective, more investigations are suggested at the field level to declare P+Zn+zeolite as the best combination for alleviation of Cd stress in different crops.

## Statements and Declarations

Ethics approval and consent to participate. We all declare that manuscript reporting studies do not involve any human participants, human data, or human tissue. So, it is not applicable.

Study protocol must comply with relevant institutional, national, and international guidelines and legislation our experiment follows the with relevant institutional, national, and international guidelines and legislation.

**Consent for publication:** Not Applicable

**Data Availability:** All data generated or analyzed during this study are included in this published article.

**Competing Interests:** The authors declare no competing interests.

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