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Phosphorus and Micronutrient Interactions in soil and their Impacts on Maize Growth

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INTERACTIONS AMONG phosphorus (P) and micronutrients may greatly influence plant growth and soil productivity. Thus, a greenhouse investigation of a complete randomized design was conducted to highlight such interactions in which a clayey soil (enriched with either 5 mg Fe kg^{-1} soil , 1mg Mn kg^{-1} soil ,or 1.5 mg Zn kg^{-1} soil) received P in the form of calcium superphosphate at three rates equivalent to 6.7 (P1), 13.4 (P2, recommended dose) and 20.1 mg P kg^{-1} (P3). Then, the soil was planted with maize seeds (*Zea mays* L var f16) for 60 days. Our results showed that application of P3, but not P2, raised significantly the fraction of P in soil which was extracted by ammonium bicarbonate- diethylene Tri amine penta acetic acid (AB-DTPA- P) versus P1. Likewise, AB-DTPA extractable Fe and Mn increased significantly in soil with increasing the rate of applied P, while AB-DTPA extractable-Zn decreased. In P-Fe interaction experiment, increasing the dose of applied P enhanced significantly maize dry weights, although did not affect significantly their heights. This is because P applications led to significant increases in Fe and K contents within plant tissues. Regarding P-Mn interactions, application of P2 significantly raised Mn content within plants while the highest application rate of P (P3) diminished this content. In spite of that, maize dry weights seemed to be comparable between P2and P3 and both exhibited higher dry weights than P1. Finally, results of P-Zn interactions revealed that both N and Zn contents significantly increased within plants due to increasing the rate of applied P fertilizer. Accordingly, plant dry weights increased significantly. In conclusion, plants that received the recommended doses of P or even less need to absorb more micronutrient (Fe, Mn and Zn) from soil for metabolism and growth; yet, high P inputs increased the uptake of Fe and Zn by plants while diminished Mn uptake.

Keywords: Fe; Zn; Mn; micronutrient availability; micronutrient uptake; interactions; maize.

1. Introduction

Phosphorus is a limiting nutrient for more than 40% of the world's arable lands (Balemi and Negisho 2012), probably because of its low solubility and mobility in soils. (Shen *et al*. 2011). It accounts approximately for 0.2% of the plant material on dry weight basis (Balemi and Negisho 2012). It is a key component in biosynthesis of nucleic acids (RNA and DNA) (Lambers *et al*. 2015), phosphoproteins, phospholipids, sugar phosphates, ATP, etc (Wieczorek *et al*. 2022).

In poor fertile soil, application of P fertilizers is an obligation to improve P availability (Shen *et al*. 2011; Habib *et al*. 2021), nevertheless, the high doses of chemical P fertilizers may immobilize other essential nutrients such as Fe (Xiaoning *et al*. 2021), Mn and Zn (Barben *et al*. 2010; Xiaoning *et al*. 2021) and this in turn lessen their uptake by plants (Zhu *et al*. 2002; Li *et al*. 2007; Montalvo *et al*. 2016).

Micronutrients are also essential for plant growth. For example, Fe is needed for enhancing the activities of many metalloproteins within plants that participate in various biological processes such as photosynthesis, and the repairing and replication of

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DNA (Schmidt *et al.* 2020; Robe *et al.* 2021; Gracheva *et al.* 2023).

Mn is an activator/cofactor for many enzymes (Lambers *et al.* 2015). It promotes vital physiological and metabolic activities such as electron transfer to photosystem II, protein synthesis, cell elongation and division (Costa *et al.* 2023). Regarding Zn, it is a cofactor for >300 proteins, including zinc finger proteins, RNA polymerases and DNA polymerases (Gupta *et al.*, 2016; Mandel and Ghosh 2021).

These three micronutrients take part directly and/or indirectly in plant nutrition while their deficiency may cause growth retardation in plants, and finally plant death (Robe *et al.* 2021; Ivanov *et al.* 2022; Ahmadi *et al.* 2023; El Amine *et al.* 2023; Younas *et al.* 2023). On the other hand, they become rapidly immobile in soils within short time periods of application (Abbas and Salem 2011; Abbas 2013; Elshony *et al.* 2019).

Possibly, introducing high inorganic P inputs affect the uptake of the abovementioned micronutrients by plants. For example, P fertilizers lessened shoot Zn while raised shoot Fe and Mn (Zhang *et al.* 2017), or may probably have little effect on micronutrient availability according to Richards *et al.* (2011). Anyhow, results of these interactions seemed to be confusing in relation with P inputs. Probably, altering inorganic P to organic forms or even chelating P via root exudates may lessen such antagonistic effects (Brinch-Pedersen *et al.* 2002; Dotaniya and Meena2015; Lambers *et al.* 2015). In this context, the rate of formation of root exudates that chelate P increases when plants suffer from shortage of P (Tawaraya *et al.*, 2014) and these exudates hence, on the other hand, mobilize micronutrients and increase their availability (Mitra *et al.*, 2020).

The current study investigates the impacts of applied P at different rates on the availability of the abovementioned micronutrients in soil and their concentrations within the tissues of grown plants. To attain, this aim, a greenhouse experiment was guaranteed to monitor precisely such interactions. Specifically we anticipate that plants that received the recommended doses of P can absorb more micronutrients from soil needed for metabolism and this enhances plant growth (hypothesis 1). Nevertheless, plants that received high inorganic P doses may suffer from micronutrients deficiency because they are partially precipitated in soil; hence their intake and distribution within plant tissues decrease considerably (hypothesis 2).

2. Materials and Methods

2.1. Material of study

A soil was collected from the top-layer (0-30 cm) of the experimental farm of the Agricultural Research and Experimental Center, Faculty of Agriculture, Benha University, Egypt (31^{o} 13′ 24.4″ E. and 30^{o}; 21′ 22.2 ″ N) to attain the aim of this study. This sample was air dried, crashed and sieved via a 2-mm sieve then analyzed for its physical and chemical properties according to Sparks *et al.* (1996) and Klute (1986), respectively and the obtained results are shown in Table 1.

Table 1. Physical and chemical properties of the soil under investigation.

Parameter	Soil physical characteristics							
	Sand (%)	Silt (%)	Clay (%)	Textural class	Field capacity (%)	Wilting point (%)	Available water (%)	$CaCO_3$ (g kg^{-1})
Value	35.9	17.3	46.8	clay	61.45	30.73	30.73	38.5

Parameter	Soil chemical characteristics						
	pH	EC (dSm^{-1})	Organic matter (g kg^{-1})	Available P (mg kg^{-1})	AB-DTPA-Fe (mg kg^{-1})	AB-DTPA-Mn (mg kg^{-1})	AB-DTPA Zn (mg kg^{-1})
Value	7.28	1.33	10.07	12.026	25.74	20.36	1.12

Soil pH* was determined in 1:2.5 soil:water suspension, while the EC** was determined in soil paste extract, available P was extracted by AB-DTPA.

The soil sample was divided into three equal portions. The first one was spiked with 20 mg Fe kg^{-1} soil ($FeSO_4.7H_2O$, Sigma-Aldrich), the second one was spiked with 10 mg Mn kg^{-1} soil ($MgSO_4. H_2O$, Reidel-de Haën) and the third one was spiked with 10 mg Zn kg^{-1} ($ZnSO_4. 2H_2O$, Sigma-Aldrich). Maize seeds (*Zea mays* L var F16) were brought from Techno Seeds Company, Alexandria.

2.2. The green house trial

Three pot experiments of a randomized complete block design were conducted under the greenhouse conditions of Soils and Water Department, Benha University. In each group, the investigated soil was spiked with either Fe, Mn or Zn then received calcium superphosphate (155g P_2O_5 kg^{-1}) at three rates i.e. 6.7 (P1), 13.4 (P2) and 20.1 (P30) mg P kg^{-1} soil corresponding to 50, 100 and 150% of the recommended P dose where each treatment was replicated 3 times.

Portions of the considered soil each of 4 kg soil was packed uniformly in plastic pots of 18 cm height ×22 cm diameter in the summer season of 2021-2022, then planted with 6 seeds of maize and received fertilizers at rates of 50 mg kg^{-1} K_2SO_4 (480 g K_2O kg $^{-1}$) and 310 mg kg^{-1} urea (460 g N kg $^{-1}$). In this context, K was added just before planting, while nitrogen was added at three equal shares i.e. just before planting, 10 days later, and 25 days after planting. Plants were then thinned to three seedlings per pot after emergence and left to grow under the greenhouse conditions for 60 days. Through this period, soil moisture was kept gravimetrically at the field capacity.

At the end of the experimental investigation, whole plants were gently removed from soil and their fresh weights were assessed Plants were then washed several times with tap water then with distilled water and left to dry in air. Thereafter, plants were oven dried at 70° C for 48 h for determination of their dry weights. Moreover, soil samples were collected from rhizosphere of each treatment (see Fig 1).

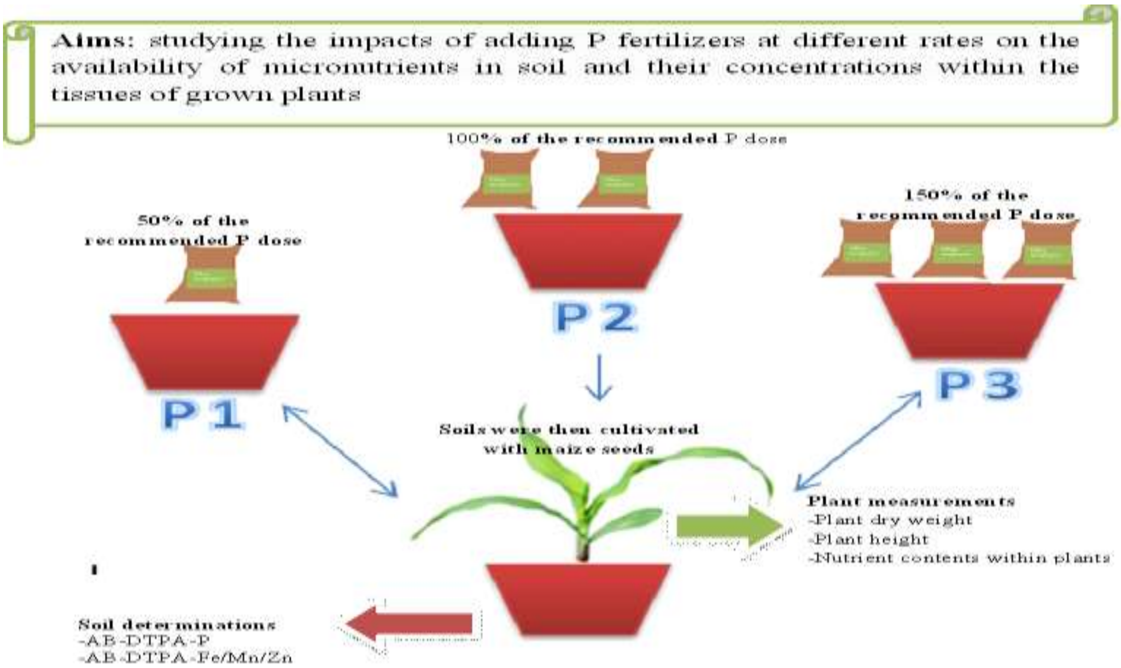

Fig. 1. Scheme of the investigation. For Abbreviations: 6.7 mg P kg^{-1} soil (P_1), 13.4 mg P kg^{-1} soil (P_2) and 20.1 mg P kg^{-1} soil (P_3).

2.3. Soil and plant analyses

Available P was extracted by ammonium bicarbonate- diethylene Tri amine Penta acetic acid (AB-DTPA) according to Soltanpour and Schwab (1977). P was then measured calorimetrically via ascorbic acid method by spectrophotometer (Spectronic 20D). Available Fe, Mn N and Zn were extracted by AB-DTPA then determined by Atomic absorption (UNICAM 929 AA spectrometer. Samples of the dried plant material were digested in a mixture of concentrated H_2SO_4 and $HClO_4$ acids on a sandy hot plate at 250 °C as outlined by Cottenie *et al*. (1982). Total phosphorus in plant digest was measured spectrophotometrically following the ascorbic acid method while Fe,Mn and Zn were determined by Atomic absorption(UNICAM 929 AA spectrometer).

2.4. Data analyses

Data was subjected to analyses of variance via SPSS 18 statistical software following one-way ANOVA and Dunken’s test. Also, Pearson’s correlation matrices were considered to evaluate the relationships among variable under investigation. Figures were plotted with Sigma Plot 10.

3. Results

3.1. Implications of interactions between phosphorus and the studied micronutrients on their available contents in soil

Application of P-fertilizer raised significantly AB-DTPA extractable P content in soil, especially with increasing its rate of application (Fig 1). Also, AB-DTPA- extractable Fe and Mn increased significantly owing to such additives. On the contrary, increasing P inputs resulted in significant reductions in AB-DTPA extractable Zn, following the sequence of $P_1>P_2>P_3$.

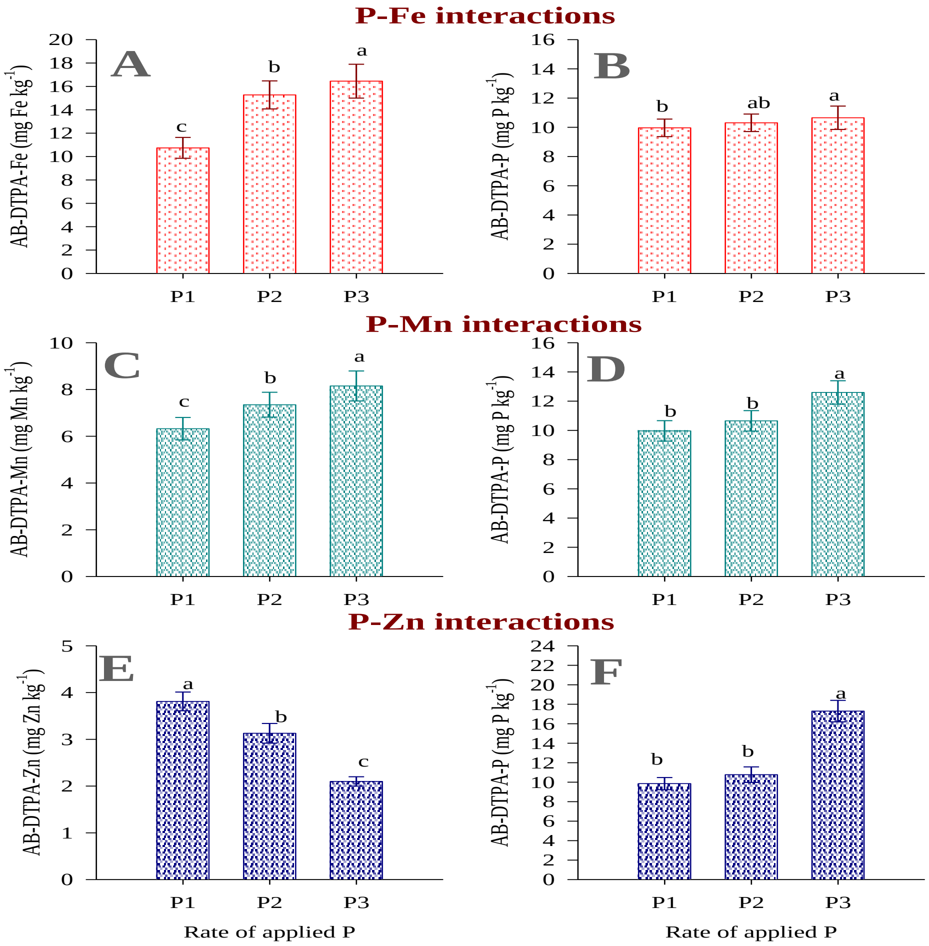

Fig. 2. Interactions between P and micronutrients in soil, namely Fe, Mn and Zn (means + st dev).For Abbreviations: 6.7 mg P kg^{-1} soil (P_1), 13.4 mg P kg^{-1} soil (P_2) and 20.1 mg P kg^{-1} soil (P_3). Similar letters indicate no significant variations among treatments.

3.2. Implications of phosphorus-micronutrient interactions on maize growth and nutrients uptake

3.2.1. Effects of P - Fe interactions on maize growth and nutrient contents within plant tissues

Application of P fertilizer raised significantly plant dry weight, especially with increasing the rate of application (P_3) while recorded no significant impacts on plant height (Fig 2). Likewise, P-inputs raised both Fe and K contents within plants, while decreased considerably N content. Surprisingly, P content did not vary significantly within plant tissues owing to increasing the rate of applied P.

Table 2 reveals that maize dry weights were correlated significantly and positively with Fe content within plant tissues, but not with P. Nevertheless, heights of maize were significantly correlated with the plant content of P. It is worth noting that AB-DTPA-Fe and AB-DTPA-P were correlated significantly and positively with each other and both influenced significantly the level of Fe content within plants. These results highlighted the positive impacts of P on increasing Fe uptake by plants, especially within plant tissues. Maybe, P was precipitated in either soil or plant; thus no significant correlations were detected between P content in plant and AB-DTPA-P.

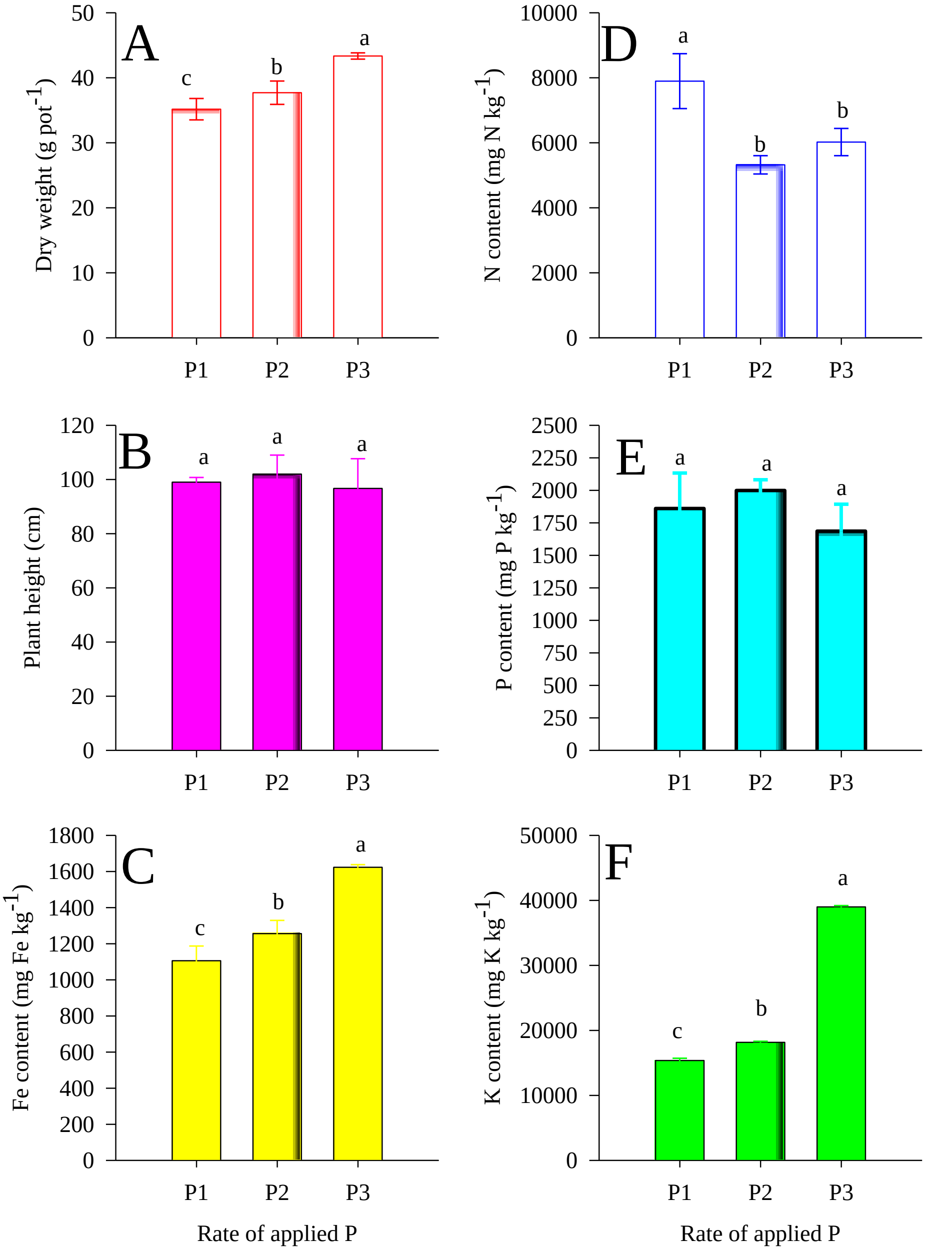

Fig. 3. Plant growth parameters and nutrient contents within tissues (means± standard deviations) as affected by P-Fe interactions. See footnote Fig 1. Similar letters indicate no significant variations among treatments.

Table 2. Maize growth parameters and its nutrient content as affected by P-Fe interactions.

	AB-DTPA-Fe	AB-DTPA-P	P-plant	Fe-plant	Dry weight	Plant height
AB-DTPA-Fe						
AB-DTPA-P	0.670^{*}					
P content in plants	0.010	0.389				
Fe content in plants	0.860**	0.693**	-0.326			
Dry weight	0.852**	0.843**	-0.122	0.972**		
Plant height	0.249	0.775*	0.868^{**}	0.092	0.319	

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

3.2.2. Effects of P - Mn interactions on maize growth and nutrient contents within plant tissues

Maize dry weights increased significantly owing to the application of either P_2 or P_3, with no significant variations among these two treatments (Fig 3). Also, application of P_2 raised significantly Mn content within plants, while P_3 diminished this content.

On the other hand, K content was not affected by P application rates while N content decreased significantly owing to the application of P_2 then raised again with application of P_3. In case of P, its content within plant tissues remained unchangeable with the application of P_2, though it decreased significantly with the application of P_3.

AB-DTPA- Mn was significantly correlated with AB-DTPA-P and both fractions affected significantly plant dry weights (Table 3). A point to note is that maize dry weights was affected significantly and positively with Mn content in plants (but not P); in spite of the existence of a significant correlation between the AB-DTPA extractable fractions of these two nutrients in plants.

Unexpectedly, no significant correlations were detected between AB-DTPA extractable fractions of both P and Mn in soil and their corresponding concentrations within plants. Probably, P and Mn interact forming insoluble salts that precipitate in either soil or within the grown plant.

Table 3. Maize growth parameters and its nutrient content as affected by P-Mn interactions.

	AB-DTPA-Mn	AB-DTPA-P	P-plant	Mn-plant	Dry weight	Plant height
AB-DTPA-Mn						
AB-DTPA-P	0.956**					
P content in plants	-0.091	-0.218				
Mn content in plants	0.508	0.276	0.702*			
Dry weight	0.873**	0.751*	0.401	0.838**		
Plant height	0.644	0.741*	0.309	0.321	0.688*	

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

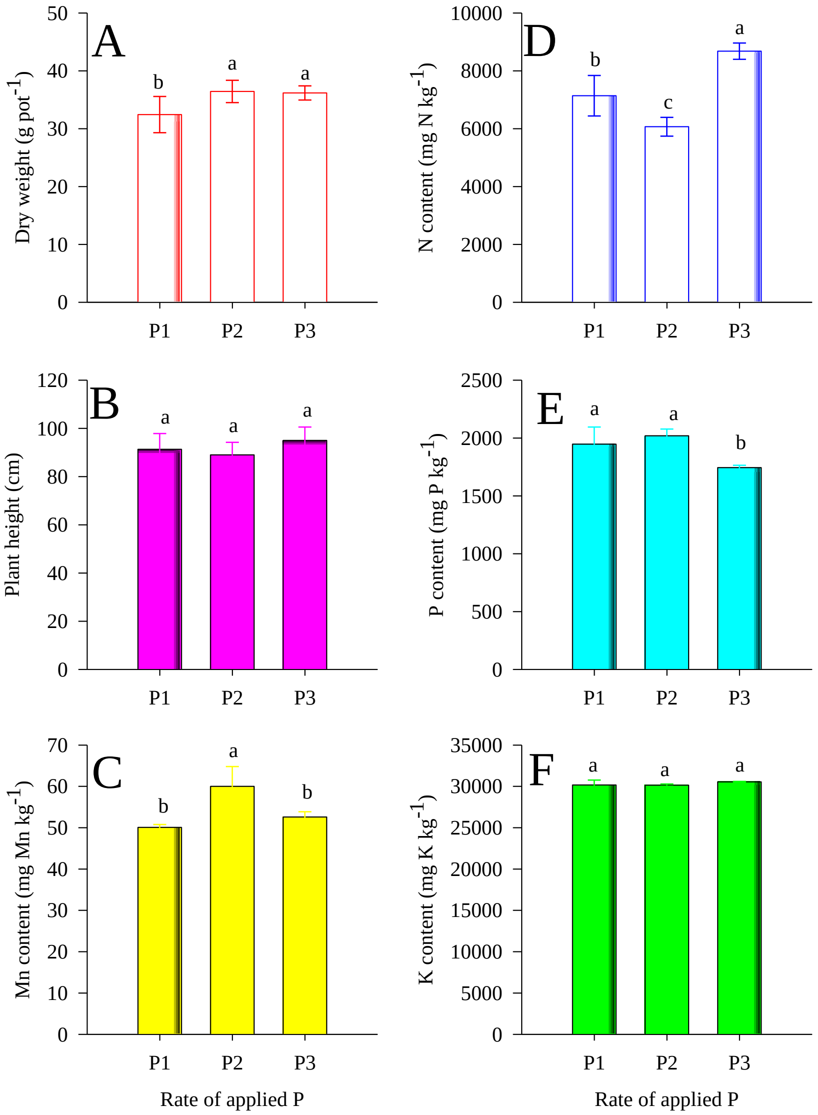

Fig. 3. Plant growth parameters and nutrient contents within tissues (mean±standard deviations) as affected by P-Mn interactions. See footnote of Fig 1. Similar letters indicate no significant variations among treatments.

3.2.3. Effects of P - Zn interactions on maize growth and nutrient contents within plant tissues

Application of P fertilizer enhanced maize dry weights; yet such increases were only significant with the application of P_3 (Fig 4). These inputs also raised Zn and N content within plants, while recorded no significant impacts on either P or K contents. It is worth noting that plant heights were also not significantly affected by increasing the rate of applied P fertilizer.

There was a significant positive correlation between AB-DTPA-Zn and AB-DTPA-P. Likewise, these two fractions were correlated significantly and negatively with the dry weights of maize plants (Table 4). Although, Zn-content in plants was correlated significantly with AB-DTPA-P; yet there was no significant correlation between Zn in plants and AB-DTPA-Zn. This might indicate that P was the dominant factor affecting Zn availability and uptake by plants. It is then thought that Zn precipitates in the form of phosphate salts in both soil and plant.

Table 4. Maize growth parameters and its nutrient content as affected by P-Zn interactions.

	AB-DTPA-Zn	AB-DTPA-P	P-plant	Zn-plant	Dry weight	Plant height
AB-DTPA-Zn						
AB-DTPA-P	-0.858**					
P content in plants	0.130	0.387				
Zn content in plants	0.547	0.779*	0.663			
Dry weight	-0.339	0.725*	0.860**	0.936**		
Plant height	0.372	-0.036	0.735*	0.563	0.649	

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

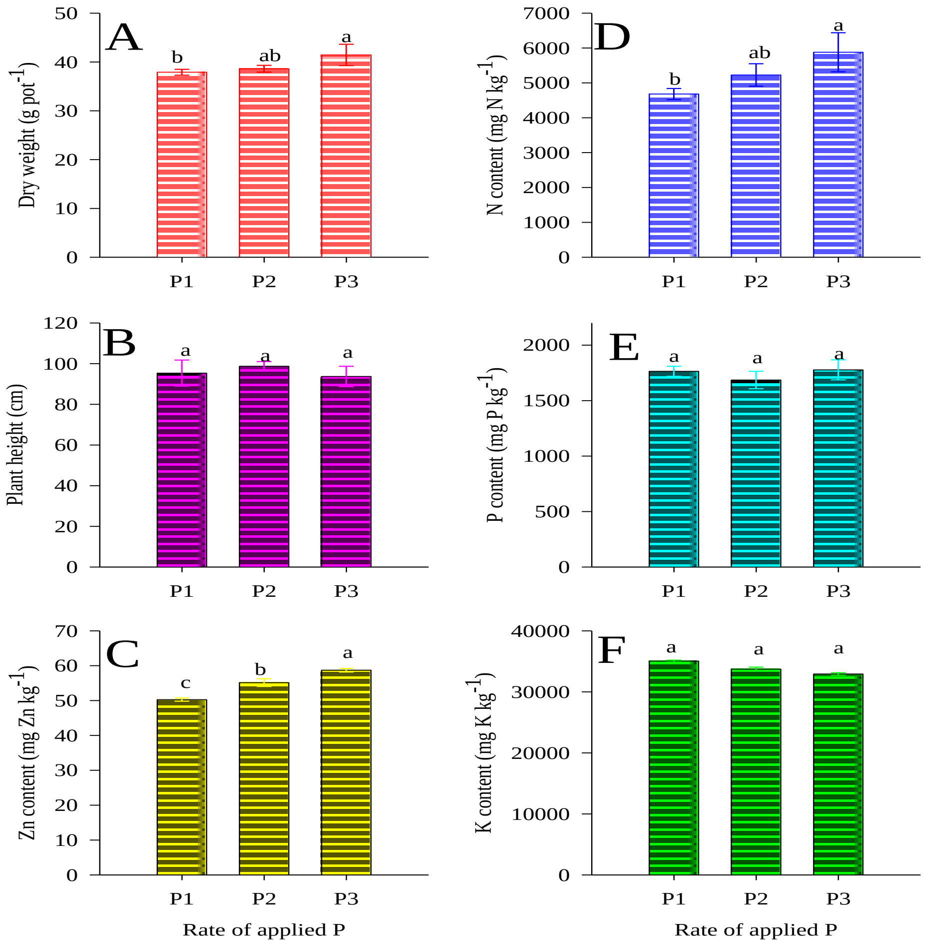

Fig. 4. Plant growth parameters and nutrient contents within tissues (mean±standard deviations) as affected by P-Zn interactions. See footnote of Fig 1. Similar letters indicate no significant variations among treatments.

4. Discussion

Application of P_3 raised significantly extractable AB-DTPA- P in soil versus the addition of P_1. Yet in case of P_2, the concurrent increases in extractable AB-DTPA- P seemed to be comparable with P_1. Maybe P underwent rapid fixation in soil to attain a new equilibrium (Filho *et al.* 2020; Weeks *et al.* 2019); nevertheless, the high P dose exceeded the fixation capacity of soil (Ibia and Udo 1993) and therefore more P became labile.

AB-DTPA extractable Fe and Mn also increased significantly in soil with increasing the rate of applied P. These results contradict those of Fageria (2001), Chatterjee *et al.* (2014) and Moharana *et al.* (2017) who recorded significant reductions in the bioavailable fractions of soil micronutrients with increasing the rate of applied P. Probably, the salt complexes which resulted from P-micronutrient interactions were not stable in soil. In other words, P-fixation is a pH dependent phenomenon and organic residues (Johan *et al.* 2021), root exudates (Zhang *et al.* 2016) and CO_2 produced via the respiration of plant roots and soil biota may decrease soil pH hence temporarily mobilize soil P (Lu *et al.* 2020; Johan *et al.* 2021). This in turn set micronutrients free. According to Ahmed *et al.* (2013), the availability of P in soil with ageing takes the shape of consecutive peaks with noteworthy increases followed by substantial reductions.

Regarding AB-DTPA extractable-Zn, our results reveal that this content decreased significantly in soil with increasing the rate of applied P. Mostly, this result was a consequence of increasing Fe and Mn in soil which compete with Zn on sorption on soil sites (Xu *et al.* 2020) and/or precipitation via applied P fertilizer (Andrunik *et al.*, 2020).

4.1. P-Fe interactions

Increasing the dose of applied P enhanced significantly maize dry weight, while recorded no noticeable impacts on plant height. Although, such increases led to concurrent significant increases in Fe and K contents within plant tissues; yet no noticeable increases were found in P content within plant tissues. This might indicate that P was a growth limiting factor (Krouk and Kiba 2021) because of its low mobility in soil (Lambers and Plaxton 2015). Thus, higher P doses enhanced plant growth rather than being accumulated in plant tissues in high concentrations (Mohamed *et al.* 2019; Abdelhafez *et al.* 2021). Concerning N, its content was high in plants when received the lowest rate of P while decreased considerably with higher P doses. This result supports the abovementioned explanation as both P and N were incorporated in plant metabolism leading to significant enhancement in plant dry weights (Abd El-Hady *et al.* 2023; Elsherpiny *et al.* 2023; Farid *et al.* 2023; Farrag and Baqr 2023; Khalil *et al.* 2023) .

4.2. P-Mn interactions

Application of P_2 significantly raised Mn content within plants while the higher application rate of P (P_3) diminished Mn content. Two scenarios might account for such reductions (1) the dilution effect owing to increasing plant growth when plants received the highest P dose and/or (2) precipitation of Mn forming stable minerals with P (Andrunik *et al.* 2020). The former explanation is accepted in case of application of P_2 because maize dry weights increased significantly when received this P dose. The latter explanation (2nd one) may be more valid to explain the reductions that occurred in Mn content within plant tissues owing to the highest P application dose (P_3). Although, P is an important nutrient for plant metabolism (Meng *et al.* 2021); yet plants also need other nutrients such as Mn (Schmidt and Husted 2019). In presence of high doses of P, Mn specification could be altered in plants (McNaughton *et al.* 2010) and therefore its translocation decreased considerably to shoots and grains. In case of N, its accumulation decreased with P_2 while increased with P_3. With application of P_2, N was mostly incorporated in plant metabolism; yet at the higher doses it is more likely to be accumulated within plant tissues rather than being assimilated in plants and this was the finding in plants that received P_3.

4.3. P-Zn interactions

Increasing the dose of applied P significantly raised plant nutritive contents, e.g. N and Zn (Fig 4). This in turn enhanced plant growth (Malhotra *et al.* 2018). Although, Zn uptake is relevant to its available content in soil (Recena *et al.* 2021); yet its balance with P is also important to avoid Zn deficiency (Santos *et al.* 2019). In our case, increasing P inputs significantly decreased AB-DTPA-Zn in soil (Fig 1), while increased Zn uptake (Fig 4). If we assume that P inputs immobilize available Zn in soil (Nichols *et al.* 2012; Saboor *et al.* 2021; Yu *et al.* 2020l), then

Zn uptake should be decreased considerably with the increasing P inputs. This is not our case. Alternatively, P additions might lead to significant increases in Fe and Mn availability in soil and this, in turn, compete with Zn on sorption sites to set it free in readily available forms that can be rapidly absorbed by plants, or otherwise lost via leaching. On the other hand, P and K contents did not vary significantly among treatments owing to the increased applied P dose. Probably, P-uptake increased with increasing the dose of P yet, it was mostly incorporated in plant metabolism and maybe underwent dilution within plant tissues.

Once inside the plant, Zn is highly mobile within xylem and phloem (Gupta *et al*. 2016), interrupting the loading sites of essential elements in roots and decreasing their translocation within plants (Rout and Das 2009). A point to note is that K is the main nutrient responsible of restoring electrolyte balance within plants (Demidchik *et al*. 2014).Since, Zn takes part in increasing plant tolerance towards drought stress and also reduces the electrolyte leakage (Umair Hassan *et al*. 2020); thus we may assume that Zn substitute K partly in some bioactivities.

5. Conclusions

Increasing micronutrients uptake by plants, namely Fe, Mn and Zn enhanced their growth, even if these plants received high P doses. This probably occurs because application of P in the form of calcium superphosphate fertilizer raised significantly the available indices of Fe and Mn in soil. Probably these two nutrients were found as impurities in P fertilizers (Cheraghi *et al*. 2012). In case of Fe, its available content increased significantly in soil with increasing the rate of P-application; consequently Fe uptake increased extensively. On the other hand, P was found at relatively low concentrations in soil versus Fe and therefore P might undergo precipitation in soil forming insoluble iron phosphate salts (Penn *et al*. 2019). This may explain the presence of insignificant relationship between P content in plant and AB-DTPA-P. Concerning Mn, it might not form insoluble stable minerals with P; nevertheless P directly interferes with Mn uptake inducing its deficiency (Pai *et al*. 2011). Accordingly plants that received high P inputs suffered from Mn deficiency and therefore increased P inputs i.e. P_3 reduced plant growth versus P2.

In case of Zn, higher P doses enhanced root elongation (Ogawa *et al*. 2014) to search for labile nutrients in soil (Li *et al*. 2016), including Zn. Root exudates then increased Zn mobility in soil (Widado *et al*. 2010) to be taken up by plants and enhance their growth. Nevertheless, P was incorporated in plant metabolism rather than being accumulated in plant tissues in high concentrations accordingly; its content did not increase significantly in plants that received higher P doses.

Based on the above findings, plants that received the recommended doses of P or even less could absorb sufficient amounts of micronutrients (e.g. Fe, Mn and Zn) from soil needed for proper metabolism and growth. This result validates the 1st assumption. On the other hand, high P inputs increased the uptake of Fe and Zn by plants while diminished Mn uptake. These results are not enough to accept or even refuse the second hypothesis and therefore more studied are needed to satisfy this point. Overall, we believe that the balanced uptake between P and micronutrients is responsible for increasing plant growth.

6. Conflicts of interest

There are no conflicts to declare.

7. Formatting of funding sources

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