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Can Humic Acid Alleviate The Adverse Effect of Elevated Phosphorus Application on Yield and Nutritive Contents of Maize Grown on a Calcareous Soil?

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GROWING maize in calcareous soils requires adequate supply of phosphorus inputs, yet excessive P-applications may affect negatively micronutrients availability in soil, including Zn. Thus, the current study evaluates the ability of humic acid (HA) to counteract the negative effects of applied P on Zn-uptake by maize plants. To achieve this aim, a field experiment was conducted in a calcareous soil ($CaCO_3$ =268.91 g kg^{-1}) following a split plot design to study the interactions between different P-inputs (100, 125 and 150% of the recommended dose, applied in main plots) and available-Zn in presence and absence of HA (applied in sub-plots at either 2 or 4 kg HA ha^{-1}). Results revealed that increasing the level of P-application raised significantly P availability and consequently its uptake by maize plants; thus, enhanced significantly plant growth parameters and grain yield. In this concern, 125% of the recommended P-dose recorded the highest partial factor productivity values. Likewise, application of HA raised significantly available-P content and improved considerably plant growth and grain yield, especially with increasing the rate of its application. On the other hand, increasing the level of P-fertilizers decreased significantly Zn-availability and uptake by plants; however, in presence of HA, this effect was counteracted. Although, this amendment recorded no significant effect on soil-Zn availability; however, it raised significantly its content in shoots but not in grains. In conclusion, HA improved significantly the productivity of maize grown on a calcareous soil. This amendment not only improved the partial factor productivity of P-fertilizer, but also increased Zn-uptake by maize plants.

Keywords: Calcareous soil; Nutrient availability; Plant uptake; Humic acid; P-Zn interactions; Partial factor productivity.

Introduction

Calcium carbonate precipitates, the major constituents in arid and semi-arid soils (Virto et al. 2018), exist mainly in micritic or microsparitic crystals (Durand et al. 2018). In Egypt, calcareous soils are wide spread in many regions of the Northern coast of the Mediterranean Sea (Morsy et al. 2019), Abu El-Matamir, El-Beheira Governorate (Abou Hussien et al. 2019), El-Nubaria region (Abdelhameid, 2020), North Sinai and Fayoum (Attia, 2019). These soils are characterized by their low available micro-nutrient contents (Karimi et al. 2020). Thus, plants grown thereon exhibit the symptoms of micronutrient deficiency unless using untraditional approaches to satisfy plant needs for nutrients such as using nano-fertilizers (Abdalla et al. 2018; El-Ghamry et al. 2018; Cieschi et al. 2019; Omara et al. 2019) and synthetic chelates (Wang et al. 2017; Rajaie and Tavakoly, 2018; López-Rayo et al. 2019). It is worthy to mention that these nano-materials may pose potential health threats when enters food chain (Zulfiqar et al. 2019). Also, synthetic

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chelates degrade comparatively slow (Freitas and Nascimento, 2017).Thus, there is an actual need to use safe ecofriendly methods to improve nutrient availability in such calcareous soils.

Maize is an important protein and calorie source for millions of people across developing countries (Saritha et al. 2020). It is also used in many food and industrial products, e.g. starch, sweeteners, oil, beverages, glue, industrial alcohol, and fuel ethanol (Ranum et al. 2014). Growing maize plants in calcareous soils requires adequate supply of phosphorus inputs (Leytem et al. 2011), yet excessive P applications may affect negatively micronutrient availability in such soils (Sims and Pierzynski, 2005), including Zn (Wang et al. 2019), consequently lessen their uptake by plants (Zhang et al. 2012). Another negative aspect of using P-fertilizers in calcareous soils is that these soluble P-fertilizers are of low use efficiency because P-inputs undergo rapid immobilization (Bindraban et al. 2020) through adsorption on the calcite surface (Izhar Shafi et al. 2020). Thus, proper management of nutritive inputs is required to sustain crop productivity grown on such soils. These nutrients not only represent a crucial issue in growing crop plants (El-Ramady et al. 2020) but also very important to meet the standards of food and fodder consumption (Vagerial et al. 2008).

One of the promising approaches to increase both P and micronutrient availability in soils is through amending soils with humic acid (HA) (Farid et al. 2018). This organic amendment may set P free in soil; hence, increase its uptake by plants (IzharShafi et al. 2020). Moreover, it may effectively increase micro-nutrient availability in soils (Olmos et al. 1998; Maruf and Rasul, 2019) probably through formation of soluble complexes (Dhaliwal et al. 2019; Boguta and Sokolowska, 2020). In case of Zn, HA probably reduces its bio-availability (Rong et al., 2020); in spite of that this organic amendment has positive impacts on Zn up-take (Horuz, 2020). It is worthy to mention that HA applications may have negative impacts on maize growth under calcareous conditions if amended at a rate of 2 g HA kg^{-1} (equivalent to 4.8 kg HA ha^{-1}) or higher (Leventoglu and Erdal, 2014). Such a find-ing requires further investigations on calcareous soils differing in both size and contents of $CaCO_3$ to maximize crop productivity grown thereon.

The current study evaluates the ability of HA to increase the productivity of maize plants grown on a calcareous soil though increasing further inputs of P-fertilizer (added as calcium superphosphate) in these soils as a necessary protocol to compensate the P fixed amounts. However, it is expected that the excessive applied P would negatively affect availability of some micro-nutritve elements e.g., Zn and consequently decrease their uptake by plant. Therefore, the current investigation involves also the application of HA which is thought to counteract the effect of the applied P and enhance the uptake of Zn by plant. Moreover, the interactions between P-inputs and soil-Zn in presence and absence of HA will be taken into account.

Materials and Methods

Materials of study

The current study was conducted in a private farm in El-Noubaria area, North West region of Egypt (30°46’ 18.7” N 29° 39’ 39.3” E). This location is characterized by a short rainy season and a long hot summer one with high relative humidity (Alnaimy et al. 2018). Prior to the experimental study, a surface soil sample (0-30 cm) was collected from the experimental field, air dried, ground and sieved to pass through a 2mm sieve. Afterwards, this sample was investigated for its physical and chemical properties as outlined by Klute (1986) and Sparks et al. (1996), respectively. The obtained results are presented in Table 1.

Humic acid (analytical grade, 99%) was obtained from Alpha Chemika Company, India. Seeds of maize (*Zea mays* L.cv. Hybrid 310) were obtained from the Agriculture Research Centre, Giza.

TABLE 1. Some physical and chemical properties of the field soil

Parameter	value
Coarse sand	12.48
Fine sand	43.66
Silt	19.27
Clay	24.59
Soil texture (USDA)	Sandy clay loam
Bulk density, Mg m^{-3}	1.41
pH	7.80
Organic matter content, g kg^{-1}	6.43
Total $CaCO_3$, g kg^{-1}	268.91
SAR	7.82
AB-DTPA extractable P, mg kg^{-1}	10.9
AB-DTPA extractable Zn, mg kg^{-1}	0.87

The experimental study

A field experiment was conducted at a private field of a calcareous nature in El Noubaria region following a split-plot design (the experimental plot was 3.5 × 3.0 m^2) with three replicates for each treatment. The different application rates of P-fertilizer (calcium super phosphate, 65.5 g P kg^{-1}) i.e., 30 kg P (the recommended dose), 38 kg P (125% of the recommended dose) and 45 kgP ha^{-1} (150% the recommended dose) were located at the main plots, while humic acid treatments (0,2 and 4 kg ha^{-1}) were placed in sub-plots. Seeds of maize were then planted at the second half of April (2019) and all plots received NK rates as recommended by the Egyptian Ministry of Agriculture i.e., 350 kg N ha^{-1} as urea (46.5 % N) and 47kg K ha^{-1} as potassium sulfate (48% K).

Common agricultural practices were followed as recommended according to the local conditions. At physiological maturity growth stage (110 days after planting), the above ground plant parts were harvested; plant growth parameters (plant height and straw biomass) as well as the gain yield and 100-seed weight were then estimated. Also, surface soil samples were collected from the rhizosphere of each treatment during plant harvest to determine the available contents of P and Zn in these samples.

Soil and plant analyses

Plant materials were oven dried at 70^o C for 48 hr, then acid digested using a tri-acid mixture (HNO_3:H_2SO_4:$HClO_4$,10:4:1) according to Sahrawat et al. (2002). The available forms of P and Zn in soil samples were extracted by ammonium bicarbonate-diethylene triaminepenta acetic acid (AB-DTPA) according to Soltanpour (1985). Total P and Zn were then estimated in both plant digests and soil extracts using ICP-MS (model ULTIMA 2).

Data Analyses

Data were statistically analyzed using PASW statistical software 18 through the analysis of variance (ANOVA) and Dunken's test at 0.05 probability level. Graphs were plotted using SigmaPlot 10 software. Phosphorus-partial factor productivity (P-PFP) was estimated according to Cassman et al. (1998) and Yadav et al. (2000) as follows:

Phosphorus-partial factor (P-PFP) = seed yield (kg ha-1)/ amount of applied P-fertilizer (kg ha-1)

Results and Discussion

Plant height and yield components of maize plants

The investigated growth parameters i.e., plant height and straw biomass as well as the grain yield increased significantly owing to increasing the level of P-application (Table 2). This is because this nutrient directly and indirectly affects plant biological processes (Raghothama, 2005; Shams and Abbas, 2019). It seems that increasing the level of P from 38 to 45 kg P ha^{-1} had no further significant effect on either plant height or straw biomass; however, such increases resulted in significant increases in grain yield. Thus, it can be deduced that P applications probably motivated maize plants grown on calcareous soils towards flowering development, rather than increasing vegetative growth. Phosphorus is considered an essential nutrient for energy-related processes in plants (Elserand Bennett, 2011) which are responsible for starch synthesis and transportation into the storage cells (Engels et al. 2012).

Application of HA also enhanced significantly the investigated plant growth parameters and grain yield, especially with increasing its rate of application, except for 100-seed weight where no significant variations were detected among treatments. This might occur because HA increased significantly nutrients availability (El-Negma, 2020) and uptake by the grown plants through forming soluble organo-metal complexes (Baigorriet al. 2019). Yet the effects of P-applications on plant yield seemed to be higher than the corresponding ones of HA. Further investigations on the implications of both P-application dose and HA on P and Zn availability and uptake would be discussed below.

Interactions among the applied doses of P-fertilizer and HA were also of significant effect (Fig. 1). In this concern, the highest increases in the investigated parameters were attained for plants that received 150% of the recommended dose of P i.e. 45 kg P + 4 kg HAha^{-1}. Such increases were about 1.9 and 1.7 folds higher than the reference treatment (the recommended dose of P +0 kg HAha^{-1}) in straw and grain yields, respectively. These results agree with those of Delgado et al. (2002), who revealed that HA applications increased the efficiency of P fertilizers.

TABLE 2. Grand means of maize height, straw and grain yields and the weight of 100 grain as affected by the applied P and humic acid

Rate of application	Plant height, cm	Straw biomass, Mg ha^{-1}	Grain yield, Mg ha^{-1}	Weight of 100-grain
		P- dose		
30.0 kg P ha^{-1}	180.33b	4.04 b	2.65 c	28.65 a
37.5 kg P ha^{-1}	194.67a	6.90 a	3.17 b	29.88 a
45.0 kg P ha^{-1}	197.67a	6.79 a	3.53 a	29.74 a
***P*-value**	8.554**	4.983*	19.979**	0.569
		Humic acid dose		
0 kg HAha^{-1}	179.33 c	5.47 b	2.67 c	29.28 a
2 kg HA ha^{-1}	190.67 b	5.96 ab	3.15 b	29.46 a
4 kg HA ha^{-1}	202.67 a	6.29 a	3.53 a	29.53 a
***P*-value**	13.572**	76.852**	18.963**	0.979

* Significant at 0.05 , ** significant at 0.01

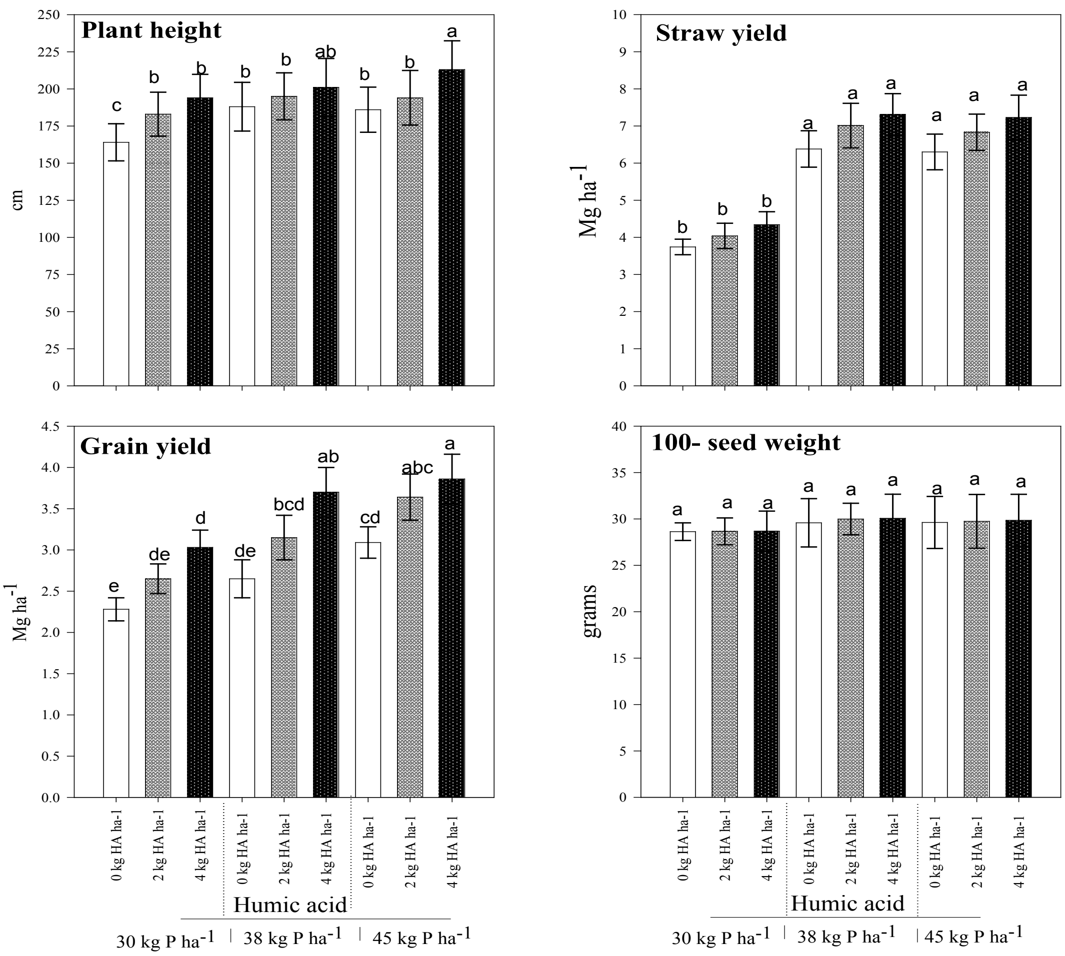

Fig.1. Plant height, straw and grain yield as well as 100-seed weight of maize plants grown on a calcareous soil as affected by applied P and humic acid. P-treatments were 30 kg P (the recommended dose), 38 kg P (125% of the recommended dose) and 45 kg P (150% the recommended dose) ha^{-1} while humic acid treatments were 0, 2 and 4 kg ha^{-1}. Different letters indicate significant variations among treatments

Concentrations of P and Zn in shoots and grains of maize

Table 3 reveals that increasing the dose of applied P (higher than the recommended dose i.e. 38 and 45 kg P ha^{-1}) raised significantly P-contents in both shoots and grains. In this concern, no significant variations were detected in P-contents within the studied plant parts among those received whether 38 or 45 kg P ha^{-1}. On the other hand, increasing the dose of P-application decreased significantly Zn contents in both shoots and grains. This might occur because of Zn precipitation in soil in the form of zinc phosphate (Wang et al. 2019). In presence of humic acid, significant increases occurred in concentrations of Zn in shoots and grains, especially in presence of the highest application level of humic acid (4 kg ha^{-1}). Likewise, humic acid raised significantly Zn contents in shoots but not in grains. Generally, Zn is an essential nutrient for proper plant growth and reproduction (Noulas et al. 2018) that stimulates the antioxidant enzymes for counteracting drought effects (Umair Hassan et al. 2020). It also plays important roles in plant defense mechanisms against pathogens (Cabot et al. 2019). It is worthy to mention that increasing the dose of P-fertilizers decreased significantly Zn content within spring wheat shoots (Zhu et al. 2001). Our results did not support such findings in maize plants that were treated with humic acid where this amendment improved significantly P and Zn contents within both shoots and grains. Probably, P-fertilizers stimulated the growth of plant roots (Gao et al. 2019) and consequently improved Zn uptake by plants (Nikbakht et al. 2008).Our findings did not also contradict those of Jones et al. (2007) who reported that application of humic acid did not affect significantly micronutrient uptake, as the authors used a low-grade commercial HA in their experimental study.

Interactions among P-treatments and humic acid were also of significant effects on both P and Zn contents in shoots and grains, except for Zn content in grains. Generally, the highest records were detected in plants that received 45 kg P+ 4 kg HA ha^{-1} (Fig 2). Although, HA raised Zn contents in shoot while this content decreased with increasing the dose of P-fertilizers. These two factors were of no significant effect on Zn content in maize grains, probably insignificant variations were due to dilution of Zn within grains that increased in yield with P fertilization as outlined by Hawkesford et al. (2012).

Availability of P and Zn in soil

Increasing the dose of applied P-fertilizer increased significantly the availability of P (AB-DTPA-P) in soil (Table 4). On the other hand, P-applications reduced significantly Zn-availability (AB-DTPA-Zn) in soil (Fig 3). This might be attributed to the precipitation of Zn in soil in the form of $Zn_3(PO_4)_2$ as shown below:

$$3Zn^{2+} + 2H_2PO_4^- + 4H_2O \leftrightarrows Zn_3(PO_4)_2.4H_2O \text{ (hopeite)} + 4H^+ \quad \text{(Lindsay, 1979)}$$

The produced hydrogen ions were then neutralized by the OH^- ions under the alkaline conditions of the calcareous soil; hence shifting the reaction towards further $Zn_3(PO_4)_2.4H_2O$ precipitations.

TABLE 3. Grand means of P and Zn contents in shoots and grains of maize plants grown on a calcareous soil as affected by the applied P and humic acid

Rate of application	P content, g kg^{-1}		Zn content, mg kg^{-1}	
	Shoot	Grain	Shoot	Grain
		P- dose		
30.0 kg P ha^{-1}	3.82b	2.38 b	25.22 b	7.75 b
37.5 kg P ha^{-1}	4.42a	2.56 a	22.32 b	7.65 b
45.0 kg P ha^{-1}	4.69 a	2.60 a	20.81a	7.57 a
***P*-value**	7.619**	5.337*	8.98**	0.146
		Humic acid dose		
0 kg HA ha^{-1}	3.80 b	2.39 b	21.06 b	7.59 a
2 kg HA ha^{-1}	4.52 a	2.56 a	22.08 b	7.62 a
4 kg HA ha^{-1}	4.60 a	2.59 a	25.21 a	7.76 a
***P*-value**	7.347**	5.284*	8.34**	0.165

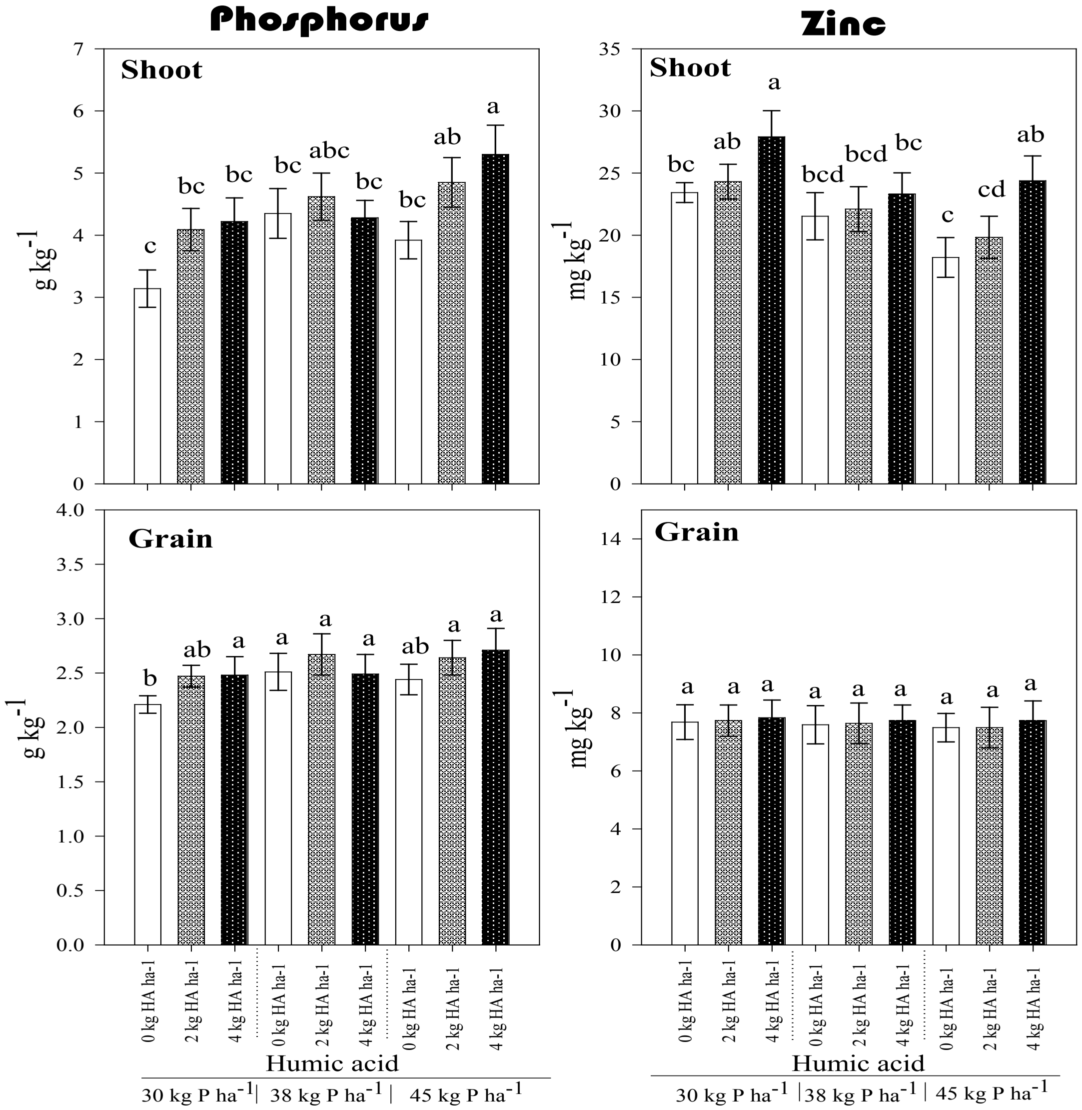

Fig. 2. P and Zn contents in shoots and grains of maize plants grown on a calcareous soil as affected by applied P and humic acid. See footnote Fig 1. Different letters indicate significant variations among treatments

TABLE 4. Grand means of P and Zn availability in the calcareous soil as affected by the applied P and humic acid

Rate of application	Available P, mg kg^{-1}	Available Zn, mg kg^{-1}
	P- **dose**	
30.0 kg P ha^{-1}	15.71 c	0.64 a
37.5 kg P ha^{-1}	19.63 b	0.53 b
45.0 kg P ha^{-1}	22.63 a	0.42 c
***P*-value**	13.514**	30.265**
	Humic acid dose	
0 kg HA ha^{-1}	16.24 c	0.55 a
2 kg HA ha^{-1}	19.66 b	0.54 a
4 kg HA ha^{-1}	22.06 a	0.50 a
***P*-value**	9.588**	1.695

Different letters indicate significant variations among treatments

Amending soils with HA increased significantly P availability in soil. Probably, humic acid (HA) has the capability to increase P- solubility in soil (Rosa et al. 2018) through binding on calcium bridges (Baigorri et al. 2019), in addition to the formation of bioavailable phosphate nanoparticles that were stabilized by humic matter (Yang et al. 2019). In case of Zn, the effect of humic acid seemed to be of no significant effect on Zn availability in soil. Two processes might take place in soil. The first one might occur through binding Zn ions on the carboxylic groups of HA (Ouyang et al. 2017; Su et al. 2019); nevertheless, HA reduced Zn bioavailability in soil (Rong et al. 2020). The second one is based on the stability of Zn-HA complex which is thought to be low because Zn is a soft metal while HA acts as a hard acid (Sparks, 2003). Thus, these complexes might set Zn free near the extended root hairs of the grown plants in exchange with H^+ ions that released by plant roots and this consequently increased significantly Zn uptake by the grown plants.

P-partial factor productivity (PFP)

Analysis of variance reveals that the values of phosphorus partial factor productivity PFP were affected significantly by the application of both P-fertilizer (F=5.657, *P*=0.012) and humic acid(F= 5.330, *P*=0.015). In this concern, the least values of PFP were recorded for the treatments that received the highest application rate of P-fertilizer (see Fig 4), while the highest values were recorded for application of 38 kg P ha^{-1} (125% of the recommended dose). On the other hand, HA raised significantly PFP in maize, especially with increasing the rate of its application, Moreover, interactions among these two factors were also of significant effect on PFP (F=4.421, *P*=0.012). In soils that received the optimum dose of P i.e., 30 kg P ha^{-1}, no significant variations were detected among PUE values owing to HA applications; however, the stimulating effect of HA was only noticeable on plants that received P-applications higher than this dose (either 38 or 45 kg P ha^{-1}).

Conclusion

Humic acid applications recorded positive impacts on maize productivity in calcareous soils. This amendment not only improved the partial factor productivity of P-fertilizer, but also increased Zn uptake by maize plants.

Author Contributions

Conceptualization, All authors; methodology, M. F. Abd El-Aziz and Ashraf M.G. Ewis; formal analysis, all authors; resources and writing—original draft preparation, all authors; writing—review and editing, all authors.

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Conflicts of Interest

The authors declare no conflict of interest

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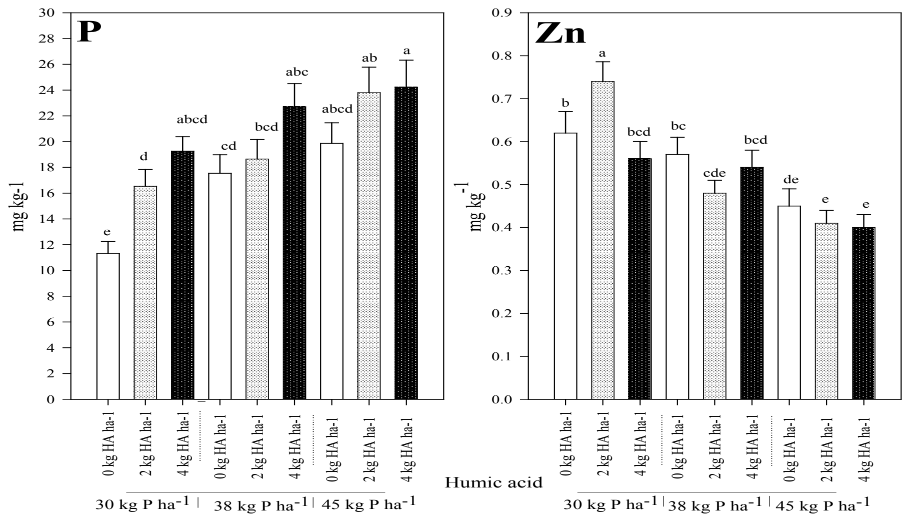

Fig. 3. P and Zn availability in the calcareous soil as affected by applied P and humic acid.
See footnote Fig. 1. Different letters indicate significant variations among treatments

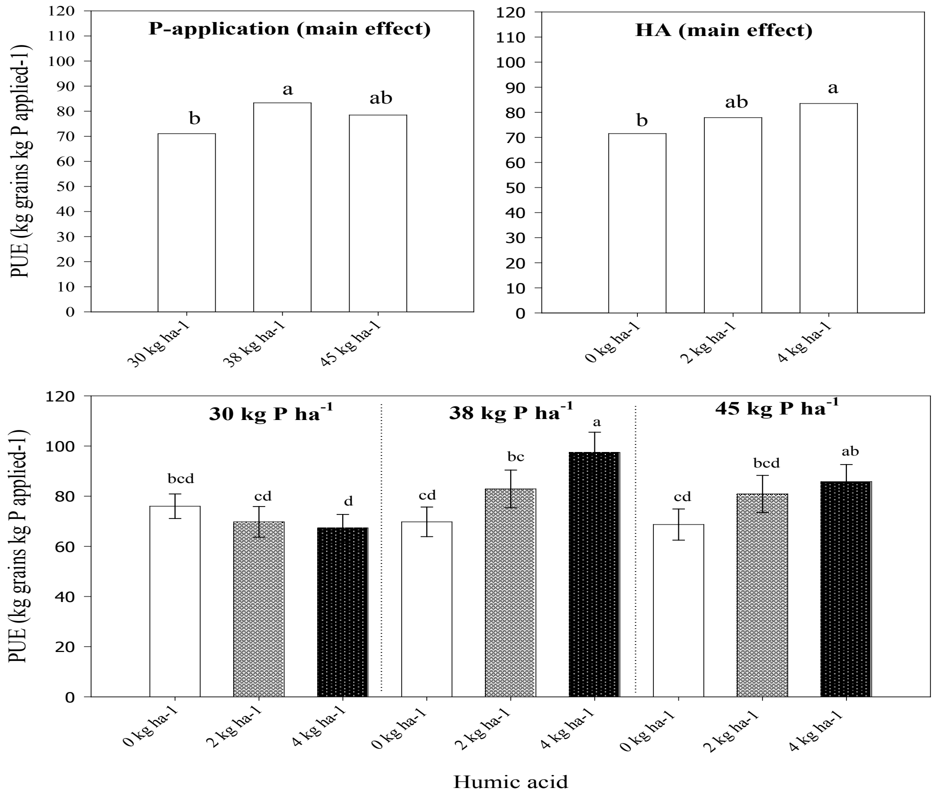

Fig. 4. Phosphorus partial factor productivity (PEP) of maize plants grown on a calcareous soil as affected by applied P and humic acid

See footnote Fig 1. Different letters indicate significant variations among treatments

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