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Effects of Excessive Application of P and Zn Fertilizer on Growth and Yield of Maize under Hydroponic Conditions

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ABSTRACT

Background: Phosphate is an essential nutrient but can be toxic to plants when phosphate accumulates in plants at high levels. The objective of the study was to find effects of excessive application of P and Zn fertilizer on growth and yield of maize under hydroponic conditions.

Methods: Research of Experiment studied from August to December 2019 at the greenhouse of the Agriculture and Aquaculture School at Tra Vinh University. The study aimed to determine the effect of high phosphate application on growth, biomass, symptoms of poisoning, phosphate and zinc absorption in maize and vegetables grown in hydroponic solution.

Result: Experimental results showed the symptoms of phosphate poisoning on maize when grown in Hoagland nutrient solution (2.0 mM P) with an additional phosphorus content of 0.1 mM P, 1.0 mM P, 3.0 mM P in the treatment with or without the addition of Zn. However, the poisoning symptoms have been yet clearly expressed when Zn was not added. Therefore, plants may exhibit phosphorus toxicity, reducing Zn absorption when the phosphorus concentration in solution was increased by more than 1%P_2O_5. It is necessary to continue investigating plants' responses to phosphate fertilizers.

Key words: Maize, Nutrient Solution, Phosphate, Zinc.

INTRODUCTION

Mineral biofortification in maize grain can be achieved through genetic or agronomic enhancements or both (Guo *et al.*, 2020); Bouis and Saltzman, 2017; Xue *et al.*, 2014). Guo *et al,* (2020) examined twenty-four maize cultivars released in China between 1930 and 2010 and found that cultivar development influenced mineral accumulation in maize grain. This research found that applying above-optimal N fertilizer significantly reduced Mn, Fe, Zn and K concentrations. Pasley *et al,* (2019) warned against the overuse of N fertilizer because of its negative impact on the soil plant balance of P, K, S, Mn and Cu. The application of high phosphorus in many crops can increase the accumulation of phosphorus in the soil, affecting the absorption of nitrogen and potassium and causing nutritional imbalance. The phosphorus content in common plants about 0.25% to 0. 6%. High phosphorus application can also cause plant phosphorus poisoning (Silber *et al.*, 2002). According to Christensen and Jackson, (1980), growing potato plants in a nutrient solution with a phosphorus content exceeding 2.22% P in the leaves showed toxic symptoms. Growing maize in a nutrient solution with a concentration of 3 mMP, the phosphorus content in the leaves was 3.39% and the yield was lower than when growing maize in a nutrient solution containing 1 mMP but showed phosphorus poisoning.

High rates of phosphorus (P) currently being applied to soils for the production of vegetables in the Mekong Delta, Vietnam, has led to concern regarding negative effects on the economy and the environment. Total P concentrations in 57% of the soil samples evaluated yielded high P concentrations (>560 mg P/kg), while 74% of the samples had high Bray 1 available P concentrations (>20 mg P/kg soil) (Thuy *et al.*, 2020). According to Minh, (1999) maize plants need a lot of macronutrients and micronutrients such as N, P, K, Mg, Bo, Cu, Zn, Mn, Fe and Mo. According to Hoa *et al,* (2008) experimenting with the effectiveness of fertilizer for hybrid maize in Tra Vinh, there was no difference in yield between the plots with fertilizer application. The yield of the P-deficient (NK) plot and K-deficient (NP) plot was high (7.64-9.77 tons/ha), equivalent to the full NPK plot (7.37 - 9.15 tons)/ha).

Maize and potato crops showed symptoms of Zn deficiency and phosphorus poisoning (Christensen and Jackson, (1980). The above treatment included Zn with levels of 0, 0.14 and 0.41 µM Zn combined with phosphate concentrations of 0.02, 0.10, 1.0 and 3.0 mM P. Maize showed symptoms of Zn deficiency. When Zn was added, plant growth was not restricted by the low phosphorus concentration in the solution. When maize plants were grown

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in a nutrient solution containing 0 mM Zn and 3 mM P, the leaf contained 3.39%P and the yield was lower than that of the treatment containing 1 mMP.

The phosphorus concentration in maize leaves was similar to that in potato plants. In the condition of Zn deficiency, raising the concentration of phosphorus in the solution increased the amount of metabolic sugar, enhanced the movement of phosphorus to the upper part and caused symptoms of phosphorus poisoning. Verticordia grass grow showed typical phosphate toxicity and inhibited plant growth in a solution with a P concentration of 3 mg/l (Siber *et al.,* 2002). According to Hawkins *et al,* (2008) when the phosphorus concentration in plants exceeded the level of 5.0 mMP on some crops, symptoms of P poisoning appeared. Therefore, the study aimed to determine the effect of high phosphorus application on growth, phosphorus and zinc absorption in maize and vegetables grown in hydroponic solution. Besides, it also aimed to investigate the harmful effects of high phosphorus application on growth, biomass and symptoms of poisoning on maize when grown in nutrient solution. There was no difference in the effect of phosphate fertilizer dosage on growth and yield maize on land but there were differences between soil types. Plants adapted and yielded better on sandy soils than on alluvial, alkaline and red soils. The total phosphorus content on fruit-bearing leaves was higher in the treatment rich in phosphorus than in the treatment with low phosphorus, but no symptoms of excess phosphorus were detected in plants (Thuy, 2023).

MATERIALS AND METHODS

Research of Experiment studied from August to December 2019 at the greenhouse of the Agriculture and Aquaculture School at Tra Vinh University. The experimental site comes under the tropical climate, situated at 9°55′23.7″N and 106°20′49.3″E long with an average altitude of 4.5 meters above sea level. The average temperature in the greenhouse was maintained from 27-29°C. The humidity of the treatments from 80-85% was controlled by a Conotec Fox-1H humidity controller.

Experimental materials

Test solution

The Hoagland nutrient solution used to grow plants was invented by Hoagland and Arnon, (1950) (Table 1). This solution had a phosphorus concentration of 2.0 mMP and 2.0 µMZn. The improved Hoagland solution contained all the mineral elements necessary for active plant growth. Concentrations of these elements were established to be as high as possible without causing salt toxicity or stress symptoms. In soil, phosphorus was at about 0.06 ppm, while in this solution, it provided 62 ppm phosphorus (2.0 Mm P) Hoagland and Arnon, (1950). Such a high starting level allows plants to be grown in a medium for a long time without additional nutrients. An essential property of Hoagland is that nitrogen is supplied in both NH_4^+ and NO_3^- forms. Supply of nitrogen in a balanced mixture of cations and anions will tend to reduce the rapid rise in pH of the medium, which is often seen when nitrogen is supplied in the form of nitrate anions. A significant issue for nutrient solutions is the maintenance of iron availability; when provided as inorganic salts such as $FeSO_4$ or $Fe(NO_3)_2$, iron can be removed from the solution under iron hydroxide. If phosphate salts are present, insoluble iron phosphate will also form. The sedimentation in the solution makes Fe always available in a state that is available to plants.

Experimental plant

The maize ‘Amazing’ (White Beard) varieties provided by ANTESCO An Giang company, cycles ranged from 50-350 days.

Experimental layout

Experiment 1

Investigate the harmful effects of high phosphorus application on biomass and symptoms of poisoning on maize plants when grown in a nutrient solution.

Table 1: Composition of improved Hoagland nutrient solution for growing plants.

Compounds	Molecular weight	Concentration of stock solution	Concentration of stock solution	Volume of stock solution 1 in liter of solution to be mixed
	g mol⁻	mM	g/L	mL
KNO_3	101.1	1,000	101.1	6
$Ca(NO_3)_2.4H_20$	236.16	1,000	236.16	4
$(NH_4)_2H_2PO_4$	115.08	1,000	115.08	2
$MgSO_4.7H_20$	246.5	1,000	246.5	1
KCl	74.55	25	1.864	
H_3P0_4	61.83	12.5	0.773	
$MnSO_4.H_20$	169.01	1.0	0.169	2
$ZnSO_4.7H_20$	287.54	1.0	0.288	
$CuS0_4.5H_20$	249.68	0.25	0.062	
H_2Mo0_4	161.97	0.25	0.04	
NaFeDTPA	558.5	53.7	30.0	0.3- 1

Experiment 2

Investigate the harmful effects of high phosphorus application on biomass and symptoms of poisoning on maize plants when grown in a nutrient solution with high phosphorus content supplemented with Zn.

The experiment was arranged in a completely randomized block design with 4 treatments and 3 replicates, including elements with phosphorus in Hoagland nutrient solution (phosphate concentration 2 mMP), including fertilizing treatments:

No	Experimental layout		
	Experiment 1	Experiment 2	
1	0.02 mMP	0.0 mMP+0 µMZn	0.0 mMP+2.0 µMZn
2	0.1 mMP	0.02 mMP+0 µMZn	0.02 mMP+2.0 µMZn
3	1.0 mMP	0.1 mMP+0 µMZn	0.1 mMP+2.0 µMZn
4	3.0 mMP	1.0 mMP+0 µMZn	1.0 mMP+2.0 µMZn

Tracking criteria

- Biomass

The biomass of maize plants after harvesting was weighed fresh by electronic balance, put in a drying cabinet with a temperature of 70°C and then weighed again in the dry weight. Maize was harvested 25 days after sowing (DAS).

- Plant analysis criteria

After harvesting, the plant samples were crushed to remove the roots and mixed well to analyze the total P and Zn content. Total phosphorus and Zn were determined by denitrification with concentrated H_2SO_4 solution and distilled water. A colorimeter determined the complete phosphorus content at 880 nm. The entire Zn content was determined by measuring on an atomic absorber.

- Indicators for water analysis

After harvesting, the hydroponic solution was re-weighed, stirred and sampled to measure EC and pH in the water.

Farming methods

Maize plants were grown in acacia resin of a volume of 4 liters until harvest (25 DAS). The seeds were sown on the support of styrofoam. The cultivation solution was Hoagland's available solution (Hoagland and Arnon, 1950). The phosphorus concentration in the solution was 62 ppm. The plastic glue was covered with black paper to limit algae growth. After one week, Hoagland solution was added corresponding to the concentrations of the above treatments. The water was added to the solution to maintain the original water level and limit the increase in salt concentration in the nutrient solution due to evaporation.

Data processing methods

Data were statistically analyzed for ANOVA (Minitab 13) using the general linear model function and the means of treatments were differentiated using Tukey's test. The level of significance used for the tests was at least 5%.

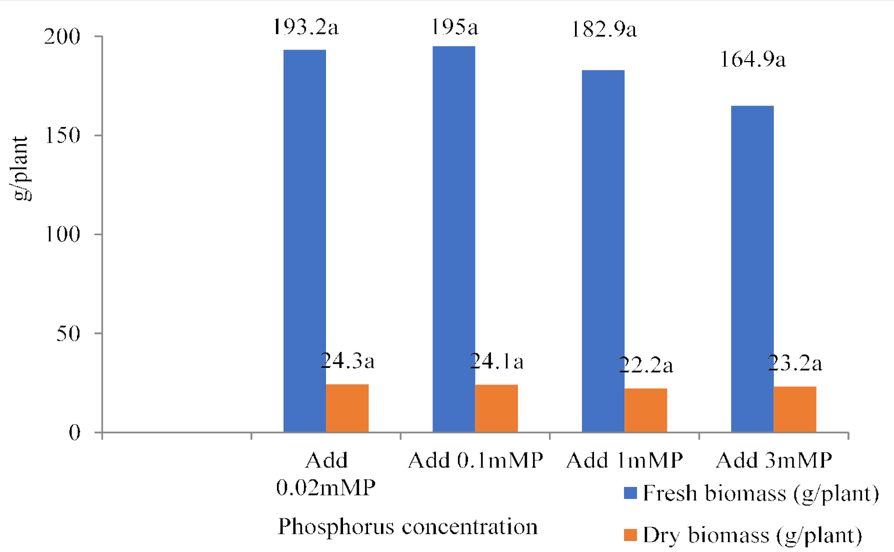
RESULTS AND DISCUSSION

Experiment 1

Investigate the harmful effects of high phosphorus application on growth, yield and poisoning symptoms on maize plants when grown in a nutrient solution.

Plant biomass in phosphorus treatments

Plant fresh biomass from 164.9-195.0 g/plant and plant dry biomass from 22.2-24.3 g/plant (Fig 1). The biomass maize in different treatments was not statistically significant.



Note: Numbers (a,b,c) with the same letter within column are not significantly different at P≤0.05.

Fig 1: Biomass (fresh and dry) on maize plants in phosphorus treatments.

Observation of growth and expression of maize plants with phosphorus fertilization treatments

In this experiment, at the early stage, when the plants were not fertilized with phosphorus in Hoagland nutrient solution with a phosphorus concentration of 2 mMP, the maize plants grew normally. However, after adding phosphate solution of 0.02 mMP, 0.1 mMP, 1.0 mMP, 3.0 mMP, symptoms such as pale yellow leaves in the leaf flesh, yellow color appearing from the yellow leaf roll to the tip of the leaf, the leaves were thinner than usual, but the veins of the leaves remained green. The above symptoms became more evident at stage 17 and 23 DAS on the leaves showing cell necrosis. The necrotic spots turned white in the two treatments with 1.0 mMP and 3.0 mMP, while the treatment with 0.02 mMP and 0.1 mMP showed the yellowing symptoms to a lesser extent (Fig 2).

Phosphate and zine content in maize

Table 2 shows a statistically significant difference in the phosphorus content of the plants in the treatments. The total phosphorus content in plant was from 0.36 to 1.51% P_2O_5 and the total Zn content in plants were from 22.23-54.9 ppm. With the concentrations of phosphorus added to the solutions, the concentration of phosphorus in the plant increased, on the contrary the concentration of Zn in plant decreased.

EC content and pH in solution after harvesting biomass

The analysis results in Table 3 show that the EC and pH indicators in the hydroponic solution were still at the threshold that allows the plants to grow well. EC content fluctuated from 0.69 ms/cm to 4.23 ms/cm, increasing gradually with the dose of phosphorus of the treatments. The EC index between 0.02 mM P low phosphorus treatment and 0.1 mM P treatment were statistically different from 1.0 mM P and 3.0 mM P high phosphorus treatment.

Experiment 2

The harmful effects of high phosphorus application on biomass and symptoms of poisoning on maize when grown in a nutrient solution with high phosphorus content supplemented with Zn.

Biomass of treatments with Zn and without Zn fertilization

Fig 3 shows that the biomass in the treatment was added 2.0 μM Zn. There was no difference in biomass between the treatments without phosphate and high phosphorus. Thus, Zn only had the effect of reducing phosphorus and phosphorus poisoning in plants. The biomass in the treatment without phosphate and Zn (150.6 g) was significantly different from that in the treatment with high phosphorus and without Zn (108 g). Biomass decreased with high phosphorus application in the absence of Zn.

Phosphate and Zn content in stems in the treatments with Zn and without Zn

In the treatments, the accumulation of phosphorus in the stem was proportional to the amount of phosphorus applied and inversely proportional to the total Zn content.

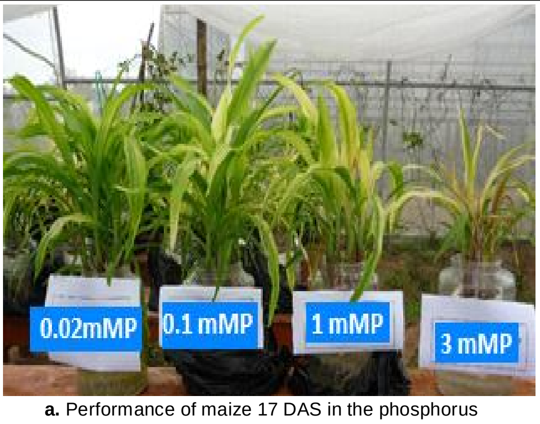
Table 4 shows the treatments without Zn. The total P content varied from (0.77 -1.34%) significantly between the 1.0 mM P treatment and the 0 mM P treatment and the total Zn content ranged from (8.16 -15.32 ppm) to a significant difference between treatments.

Table 5 shows that in the treatment with added Zn, the amount of phosphorus applied was positively correlated with the accumulated phosphorus content and negatively correlated with the Zn content in the plant. The total P higher

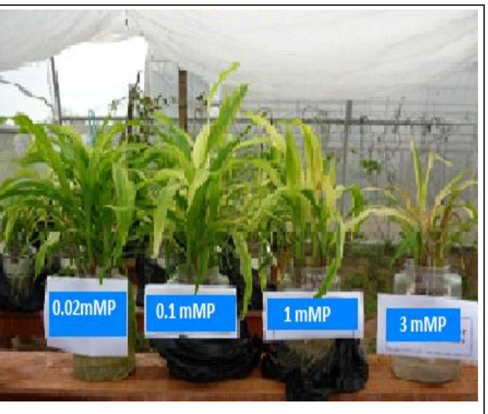
Table 2: Total P, Zn concentrations in plant samples.

Experiment	Analytical indicators	
	P total (% P_2O_5)	Total zn (ppm)
0.02 mMP	0.36^b	54.90^a
0.1 mMP	0.71^b	47.57^a
1 m MP	1.16^{ab}	29.40^b
3 m MP	1.51^a	22.23^b
CV (%)	4.55	12.95

Note: Numbers (a,b,c) with the same letter within column are not significantly different at P≤0.05.



a. Performance of maize 17 DAS in the phosphorus treatments



b. Performance of Maize at harvest in the phosphorus treatments

Fig 2: Experiment 1.

content varied from (0.97 - 1.85%) significantly between the 1.0 mM P treatment and the 0 mM P treatment and the entire Zn content varied from (8.35 -19.33 ppm) to significant difference between treatments.

Observing the growth expression of maize plants

Fig 4 shows that in the treatments without phosphate added 0 mMP and with low phosphorus 0.02 mM P, plants generally showed less yellowing in leaves than the other treatments whether with or without Zn was added.

EC content, pH in water in the treatment with Zn and without Zn

Table 6 shows the treatments with Zn or without Zn. The differences in pH values between the treatments were significant. Adding Zn and not applying Zn were not statistically significant in each pair of treatments. EC values increased when adding phosphorus in both treatments with Zn and without Zn. The difference in EC values was not statistically significant in treatments with and without Zn. The pH and EC values were high but did not affect the growth of plant roots. In the treatments, adding Zn into the solution did not affect the fluctuation of EC and pH values, but the EC and pH values fluctuated due to the amount of phosphorus applied.

Fig 1 shows that the biomass maize in different treatments was not statistically significant. The cause may be that the maize has provided enough nutrition from the Hoagland solution. Villwock *et al,* (2022) showed that complete and reduced N fertilization did not differ significantly in dry matter yield; only the level without fertilizer was considerably lower. According to the research results of Christensen and Jackson, (1980) maize plants grown in a nutrient solution with an additional phosphorus content of 3.0 mM P and 1.0 mM P showed that the yield in the 3.0 mM P treatment was lower than in the 1.0 mM P treatment, although the expression toxicity. Hoa *et al,* (2008) experimented with investigating the effectiveness of fertilizers in using the specialized fertilizer method for hybrid maize in Tra Vinh province, Vietnam. Although there was no difference in yield between the plots with fertilizer application phosphate and phosphate-free plots, the response of Maize to P and K fertilizers was deficient. The results of P-deficient (NK) and K-deficient (NP) plots were high (7.64 - 9.77 tons/ha), equivalent to the entire NPK batch (7.37 - 9.15 tons) /ha).

The leaves showed cell necrosis when phosphorus was added with 1.0 mM P and 3.0 mM P. The necrotic spots turned white while in the treatment with 0.02 mM P and 0.1 mM P. The yellowing symptoms were shown but to a lesser extent than the treatments with 1.0 mM P and 3.0 mM P. Fig 3 and 4 show the symptoms of phosphorus poisoning

Table 3: EC parameters and pH in solution.

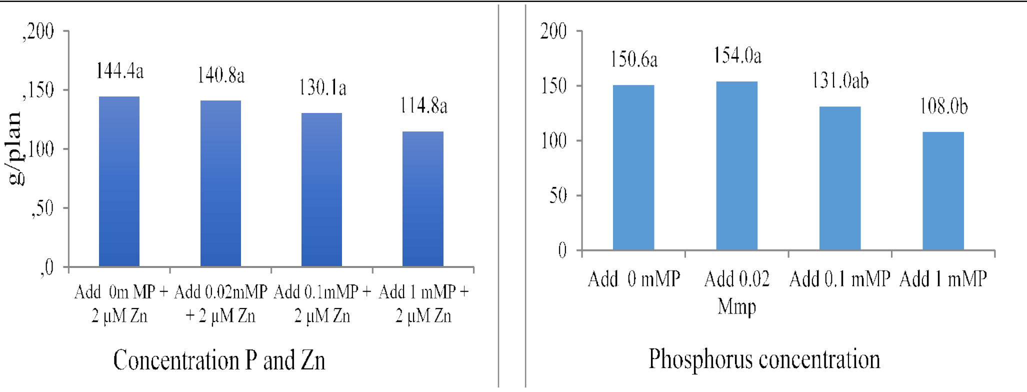
Experiment	Analytical indicators	
	EC (ms/cm)	pH
0.02 mMP	0.69^{c}	7.10^{a}
0.1 mMP	0.72^{c}	7.26^{a}
1 mMP	1.66^{b}	7.01^{a}
3 mMP	4.23^{a}	6.15^{b}
CV (%)	2.07	0.51

Note: Numbers (a,b,c) with the same letter within column are not significantly different at P≤0.05.

Table 4: Total P, Zn concentrations in the stem in the treatment without additional Zn.

Experiment	Analytical indicators	
	P_2O_5 total (%)	Total Zn (ppm)
0 mMP + 0 µMZn	0.77^{b}	15.32^{a}
0.02 mMP + 0 µMZn	0.78^{b}	15.32^{a}
0.1 mMP + 0 µMZn	1.28^{b}	8.16^{a}
1 mMP + 0 µMZn	1.34^{a}	10.9^{a}
CV(%)	3.84	2.0

Note: Numbers (a,b,c) with the same letter within the column are not significantly different at P≤0.05.



a. Biomass of maize with P+Zn

b. Biomass of maize with P+0 Zn

Note: Numbers (a,b,c) with the same letter within the column are not significantly different at P≤0.05.

Fig 3: Biomass on maize plants in treatments phosphorus with and without Zn applications.

on maize leaves. Maize leaves showed symptoms of phosphate poisoning similar to the experiment of Christensen and Jackson, (1980) growing maize in nutrient solution at a concentration of 3.0 mM P. This result shows that phosphorus content in plants greater than 1% P_2O_5 basic plants was poisoned and affected plant growth. Verticordia grown in a solution with a low P concentration of 3.0 mg/l showed typical phosphate toxicity and inhibited plant growth. There is a positive correlation between plants' phosphorus concentration, hexose phosphate in leaves and symptoms of phosphorus toxicity.

In Table 3 and 6, the EC and pH indicators in the hydroponic solution were still at the threshold that allowed the plants to grow well. The reason was that adding phosphate $(NH_4)_2H_2PO_4$to the higher concentration was more likely to precipitate PO_4^- salts, so the EC concentration increased. When the EC value grew, the pH tended to decrease. There was a statistically significant difference between treatments. The reason was that the plant thrives, so the ability to absorb nutrients is much more, so it secreted acid, which increased the amount of H^+, so the pH decreased.

Biomass decreased when phosphate was added in the absence of Zn (Fig 2). The results show that the Zn absorption decreased as the phosphorus concentration in the solution increased. According to Minh, (1999) the Zn content in maize plants was sufficient from 21 ppm to 70 ppm. Therefore, it can be seen that there is no Zn deficiency in the plant and the symptoms of leaf yellowing and necrosis on the plant are due to phosphorus poisoning in maize. The results of this study are also consistent with the study of Loneragan *et al,* (1979) when growing maize in solution with Zn concentration higher than 40 µg/g and high phosphorus appeared. Research results of Hawkins *et al,* (2008) also show that when the phosphorus concentration in the plant exceeded the allowable threshold, the total Zn content in the plant was reduced.

Observing the expression of maize plants, the plant showed signs of phosphorus poisoning and yellowing in young leaves at the 15 DAS stage. After that, the severity gradually increased to 25 DAS stage in phosphate fertilizer treatments. However, when the plants were supplemented with Zn, the phosphate poisoning expression was lower than that of the treatments without Zn and only showed phosphate poisoning in the two treatments, 0.1 mM P and 1.0 mM P. Therefore, it indicates that the addition of Zn can alleviate phosphorus poisoning. The cause may be due to increasing the level of available phosphorus (P) in soil often aggravates zinc (Zn) deficiency in plants growing on Zn-poor soils (Loneragan and Webb, 1993; Marschner, 2012; Zhang *et al,* 2012). Numerous factors have been cited for causing this harmful P-Zn interaction. It includes the impact of P on mycorrhizal colonization of plant roots (Ryan *et al.,* 2008; Marschner, 2012; Ova *et al.*, 2015) or the increased Zn-

Table 5: Total P, Zn concentrations in stems in the treatment with additional Zn.

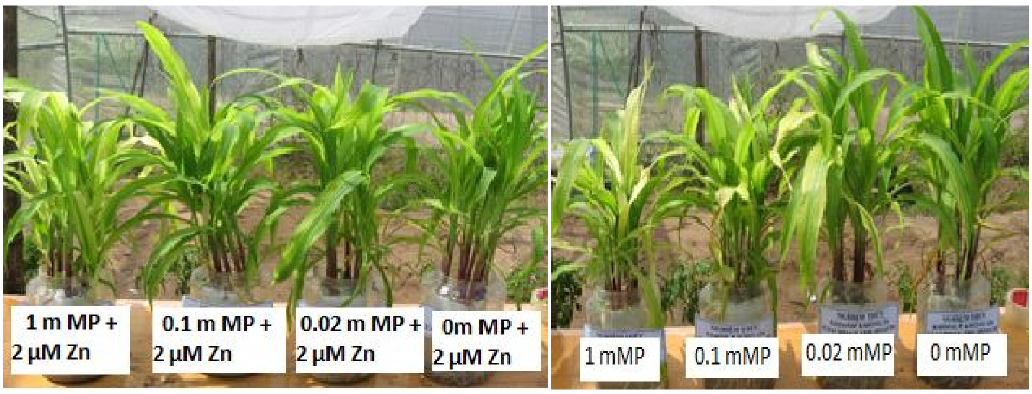
Experiment	Analytical indicators	
	P_2O_5 total (%)	Total Zn (ppm)
0 mMP + 2.0 µMZn	0.97^b	19.33^a
0.02 mMP + 2.0 µMZn	0.88^b	18.14^a
0.1 mMP + 2.0 µMZn	1.22^b	8.35^a
1.0 mMP + 2.0 µMZn	1.85^a	12.06^a
CV(%)	2.1	2.0

Note: Numbers (a,b,c) with the same letter within column are not significantly different at $P \leq 0.05$.

Table 6: EC, pH in water in treatments without Zn and with added Zn.

Experiment	0 µM Zn		2.0 µM Zn	
	pH	EC	pH	EC
0 mMP	8.01^a	2.01^b	8.23^a	2.45^b
0.02 mMP	8.34^a	2.09^b	8.19^a	2.19^b
0.1 mMP	8.33^a	1.85^b	8.32^a	2.33^b
1.0 mMP	7.36^b	3.4^a	7.54^b	3.09^a
CV (%)	0.59	4.7	0.25	2.78

Note: Numbers (a,b,c) with the same letter within column are not significantly different at Pd"0.05.



a. Performance of Maize at harvest in the Zn treatments

b. Performance of Maize at harvest in the treatments without Zn

Fig 4: Experiment 2.

binding capacity of root cell walls brought on by high P levels, which prevents Zn from moving to the upper parts (Loneragan and Webb, 1993).

Different causes have been invoked for this negative P-Zn interaction, including the effect of P on mycorrhizal colonization of plant roots (Ryan *et al.,* 2008; Marschner, 2012; Ova *et al.*, 2015) or the increased Zn-binding capacity of root cell walls induced by high P levels, which prevents its translocation to the upper parts (Loneragan and Webb 1993; Marschner, 2012).

CONCLUSION

At the concentration of phosphate fertilizer added to Hoagland's nutrient solution (2.0 mM P/l) from 1.0 mM P to 3.0 mM P, plants showed symptoms of phosphate poisoning, severe yellow leaves on young leaves and growing from leaf cover to leaf stem. Phosphorus content in plant poisoning plants is 1% P_2O_5. At high concentrations of phosphate fertilizer application from 1.0 mM P to 3.0 mM P, Zn absorption decreased. Maize plants were supplemented with 2.0 μM Zn and the phosphate poisoning expression was lower than the treatments without Zn. Continue to experiment with the land crop with many successive crops to confirm the crop's response to phosphate fertilizers on soils with high phosphorus content so that appropriate recommendations on phosphorus content for maize can be made.

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Data availability

Upon a reasonable request, the data used to support the study's conclusions are made available by the corresponding author.

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Conflicts of interest

The author declare no conflict of interest.

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