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ORIGINAL ARTICLE

EFFECT OF ADDITION DATE OF PHOSPHORUS, ZINC, ZINC SOURCE AND BIO-INOCULATION ON THE GROWTH OF MAIZE (*ZEAMAYS* L.)

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Abstract: Pots experiment was carried out at the Department of Soil and Water Resources at the College of Agriculture in the University of Anbar, Iraq during 2017 season to investigate the comparison of phosphorus, zinc and zinc source addition with and without bio-inoculation and its impact on the maize (*Zea mays* L.) growth. Treatments included the addition of mineral zinc and phosphorus at cultivation (Zn+P), the addition of mineral zinc at cultivation with phosphorus after 30 days of cultivation (Zn+P30), the addition of mineral zinc after 30 days of cultivation Zn30 and the addition of phosphorus at cultivation (Zn30+P), the same previous treatments were added using the source of chelating zinc ZnDTPA instead of mineral zinc as ZnD+P30, ZnD30 + P and ZnD+P. These aforementioned treatments were added with and without the bio-inoculation. The net number of treatments were 12 distributed according to the Complete randomized design (CRD) with three replications. Results showed the superiority of addition of mineral and chelating zinc after 30 days of cultivation (Zn30+P and ZnD30+P) and the addition of phosphorus at cultivation with inoculation and without it (Zn_p+P) in the lengths of the shoot and root total weight, zinc concentration and content in the vegetation and root yield, the concentration of phosphorus and zinc in the soil, as well as the treatment of the addition of mineral and chelating zinc at cultivation and phosphorus after 30 days of cultivation with inoculation and without it in the absorption of phosphorus.

Key words: Addition date of phosphorus, Maize, Bio-inoculation, Zinc source.

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1. Introduction

Generally, Iraqi soils suffer from a decrease in the proportion of organic matter, high content of carbon minerals, and soil pH. These factors lead to a decline in the availability of most nutrients in the soil, ineffectiveness and inactiveness of adding some nutrient sources when added as fertilizers such as phosphate and mineral fertilizers that contain micronutrients even if added with a dissolved composition in water for the speed of their interaction with components and turning them into low-melting compounds in soil solution. Therefore, the less likely they are absorbed by the plant and the more time they are added, the more difficult to dissolve.

From the studying the zinc over world, Sillanpaa

(1982) indicated that Iraqi soils were considered as a low zinc content, out of 30 soils from different countries. The researcher said in study that soil samples taken from different parts of Iraq showed that all of them suffered from a shortage of zinc to a degree that there was hardly any difference between them. He also stated that the most important problems in Iraqi soils are the high salt content, followed by increased soil content of lime (15-35% CaCO₃), which leads to the stabilization of phosphorus and therefore its unavailability to the plant, the same as in the case of zinc.

Phosphorus deficiency is a limiting factor of plant growth and production in soils that contain high levels of calcium carbonate, which in turn reduces the solubility of phosphorus. Due to limited nutrient

availability, these soils suffer from a deficiency of phosphorus and zinc, various studies have shown that adding phosphorus and zinc together at cultivation is more effective for improving crop growth and yield compared to post-cultivation. Whereas the addition of fertilizer after cultivation was more useful than at cultivation [Liu *et al.* (2016)].

Root and microbiology with soils interactions play a key role in increasing the phosphorus use efficiency, Several techniques had been used, including providing nutrients indirectly by stimulating soil-beneficial microbiology to release nutrients [Tong *et al.* (2015)]. The inoculation of seeds with plant growth-promoting rhizobacteria (PGPR) increases the plant's absorption of many elements such as calcium, potassium, iron, copper, manganese and zinc, caused by the reduction of the pH soil [Mantelin and Touraine (2004)]. This leads to increased nutrient solubility. Some species of bacteria have been found to have the potential to increase the availability of zinc by releasing it from an organic acid or dissolving it from zinc mineral compounds through chelating and then dissolving it [Hussain *et al.* (2015)]. The study aims to determine the effect of the addition date of phosphorus and zinc, as well as the effect of zinc source and bio-inoculation on the growth of maize crop.

2. Materials and Methods

pots experiment was carried out in the Department of Soil and Water Resources, College of Agriculture in the University of Anbar, Iraq. Soil samples were collected from 0-15 cm depth, taken from the fields of the College of Agriculture. They were air-dried and passed through a 4 mm diameter sieve with and well mixed. A sample was taken to study physical and chemical properties. It passed through a 2 mm diameter sieve. Table 1 shows some physical and chemical soil properties.

The source of phosphorus was Mono Ammonium Phosphate (MAP) (21%P) $(\text{NH}_4)_2\text{H}_2\text{PO}_4$. The source of zinc was mineral-sourced hydro zinc sulfate $(\text{ZnSO}_4 \cdot 7\text{H}_2\text{O})$ and zinc in the chelation formula Zn-EDTA. The experiment treatments included the following additions: mineral zinc plus Phosphorus at cultivation $T_1 = (\text{Zn}+\text{P})$, mineral zinc at cultivation plus phosphorus after 30 days of cultivation $T_2 = (\text{Zn}+\text{P}30)$, and finally the mineral zinc after 30 days of cultivation plus the addition of phosphorus at cultivation

Table 1: Some physical and chemical properties of the study soil.

Trait	Unit	Value
Texture	–	Silty Clay Loam
Sand	g.kg^{-1}	190
Silt	g.kg^{-1}	434
Clay	g.kg^{-1}	376
pH	–	7.49
ECe	dS m^{-1}	5.76
CaCO_3	g.kg^{-1}	273
OM	g.kg^{-1}	12.9
AB-DTPA extractable Zn	mg kg^{-1}	0.29
Olsen P	mg kg^{-1}	8.6
Available-N	mg kg^{-1}	143
Available-K	mg kg^{-1}	180

$T_3 = (\text{Zn}30+\text{P})$, the same previous treatments were added using the chelation zinc source ZnDTPA to become: $T_4 = \text{ZnD}+\text{P}$, $T_5 = \text{ZnD}+\text{P}30$ and $T_6 = \text{ZnD}30+\text{P}$, respectively. These six treatments were added with and without the bio-inoculate to bring them to 12 treatments. The level of zinc added from the mineral or chelated source was 5 mg.kg^{-1} and phosphorus have been added at the level of 30 mg P.kg^{-1} soil.

In the experiment, *Pseudomonas fluorescence* bacterial isolate (BIS) was used (Soil Biology Laboratory, Ministry of Science and Technology Baghdad, Iraq). BIS was activated and then incubated with peat by 1:1 ratio and then seeds of maize (Baghdad 3 cultivar) were inoculated with this BIS by 10:2 ratio (weight to weight), after this, the seeds were washed with distilled water and treated with sodium hypochlorite. Then they washed several times with distilled water again. After that, the sugar solution spread on the seeds with 20% (weight to the size). The non-inoculated maize seeds were mixed with peat and sterilized sugar solution after washing with distilled water. Pots have filled with 10 kg of soil per pot, then 5 seeds were implanted in each one and then thinned to 3 plants after a week of germination.

Nitrogen and potassium fertilizers were added to all treatments at 20 and 25 mg.kg^{-1} of urea $(\text{NH}_2)_2\text{CO}$ of 46%N (with calculating the amount of nitrogen added with phosphate fertilizer) and potassium sulfate K_2SO_4 (K_2O 50%). Potassium was added at cultivation, while nitrogen fertilizers were divided into three equal parts, the first was at cultivation, the second was after a month

of cultivation and the third was at silk threads emergence. While phosphorus and zinc were added according to experiment treatments, bio-inoculation treatments and the fertilization were arranged according to the Complete randomized design (CRD) with three replications.

All plastic pots were irrigated from one water source to maintain their moisture content to the field capacity limits throughout the experiment period as it was irrigated according to the weighted method to reach the field capacity as the pots were weighed daily to compensate the moisture loss every day. plants were harvested (vegetative weight) after 60 days of germination. Then it is immediately followed by separation of roots and washed them with distilled water to get rid of the soil, lengths of plants and roots have been measured by using the measuring tape, then the plant and roots samples were washed again with distilled water and dried in the oven at a temperature of 65°C for a period of 72 hours. The weights were estimated by using a sensitive balance. The samples were digested using wet acid-blended ($\text{HNO}_3 + \text{HClO}_4$) digestion by 1:2 ratio to estimate phosphorus and zinc on a spectrophotometer and atomic absorption devices, respectively [Jones and Case (1990)].

Root samples were collected from each experimental unit and phosphorus was estimated using the Olsen method [Olsen and Sommers (1982)], zinc was calculated by extracting it with DTPA as in the method of Soltanpour (1985). After the data were tabulated for the studied properties, the significant differences between means were compared using the Least Significant Difference (LSD) test at a probability level of 0.05 and using GenStat program 2012.

3. Results

3.1 Plant growth and Yield

The addition date of P, Zn, Zn source and BIS had a significant effect in the lengths, shoot dry weights, and total root weight. It should be noticed from Table 2 that the addition of Zn+P30, Zn30+P, ZnD+P, ZnD30+P, and ZnD+P30 achieved the highest values in the lengths and dry weights of the vegetative and total roots.

Despite there are no significant differences between zinc sources T_2 and T_3 , the chelated source of zinc T_6 with BIS achieved the highest values of the studied properties, their values were 32.6, 88.0, 24.3, and 25.9 for the length of the roots and vegetative yield,

weight of roots and shoot total weight, respectively. The least values of these studied indicators were at P + Zn addition with and without BIS inoculation, which was 24.0, 60.0, 10.3 and 16.9 for the length of the roots, vegetative total yield, root weight and shoot total weight, respectively. The BIS had a significant effect on the same indicators when adding Zn+P at cultivation. Their values were 29.0, 72.7, 11.3 and 18.9, respectively.

3.2 Phosphorus in vegetative and root weights

The results in Table 3 indicate that the effect of the addition of P, Zn, Zn source and BIS has a significant effect on the concentration and content of phosphorus in the shoot and root total weight. It should be noticed from the table that the addition of Zn+P30, Zn30+P, ZnD+P, ZnD30+P and ZnD+P30 achieved the highest values in the concentration of phosphorus and its content in the vegetative and root total weight, although there are no significant differences among the sources of zinc after or before the addition of phosphorus. The ZnD+P30 with the presence of BIS achieved the highest values for the studied properties, the values were 0.97, 2.2, 13.83 and 57.20 for both the concentration of phosphorus in total roots and shoots, the phosphorus content in the root and vegetative weight successively.

The least values for the studied indicators were noticed when Zn + P were added together with or without BIS inoculation. Their values were 0.79, 1.2, 8.13 and 20.27 for phosphorus concentration in the roots, shoots total weight, phosphorus content in roots and shoot total weight indicators respectively. The BIS inoculation had a significant effect in the same indicators when adding zinc with phosphorus at cultivation and their values were 0.93, 1.7, 10.96 and 32.30, respectively.

3.3 Zinc in vegetative and root weights

The results in Table 4 show that the addition of Zn+P30, Zn30+P, ZnD+P, ZnD30+P, and ZnD+P30 achieved the highest values in Zn concentration and content in the total vegetative and root weight of the plant. Although, there were no significant differences between zinc sources before or after the addition of phosphorus, the chelated source of zinc when added after phosphorus and with the presence of the BIS achieved the highest values for the studied properties, with values of 19.1, 15.6, 270.3 and 399.7 for both zinc concentration in the root and shoot total weight, and the zinc content in the total vegetable weight. The least values for these indicators were when Zn+P were

Table 2: The effect of experimental treatment on the dry lengths and weights of the vegetative and root compound of the maize plant.

Inoculation	Fertilizer treatments	Length (cm)		Dry biomass (g pot ⁻¹)	
		Root	Shoot	Root	Shoot
Without inoculation	Zn+P	24	60.3	10.4	16.9
	Zn+P ₃₀	26.3	64	11.9	18.8
	Zn ₃₀ +P	25.3	67.3	10.7	19.8
	ZnD+P	27.3	71.3	12.1	21
	ZnD ₃₀ +P	28.6	77	12.5	23.8
	ZnD+P ₃₀	29.3	72.6	13.2	22.0
With inoculation	Zn+P	29	72.7	11.8	18.9
	Zn+P ₃₀	30	74.7	13.3	21.4
	Zn ₃₀ +P	29.6	77.6	12.2	22.3
	ZnD+P	30.3	80.6	13.8	22.8
	ZnD ₃₀ +P	32.6	88	14.3	25.9
	ZnD+P ₃₀	31.6	83.3	13.9	24.2
L.S.D(0.05)		2.22	4.15	0.68	0.90

Table 3: The impact of experimental treatments on phosphorus concentration and content in the vegetative and root total weight.

Inoculation	Fertilizer treatments	Phosphorus concentration (mg.g ⁻¹)		Phosphorus content (mg pot ⁻¹)	
		Root	Shoot	Root	Shoot
Without inoculation	Zn+P	0.79	1.2	8.13	20.27
	Zn+P ₃₀	0.72	1.3	8.50	24.47
	Zn ₃₀ +P	0.82	1.4	8.73	27.70
	ZnD+P	0.85	1.4	10.23	29.53
	ZnD ₃₀ +P	0.84	1.5	10.40	35.17
	ZnD+P ₃₀	0.87	1.6	11.46	35.87
With inoculation	Zn+P	0.93	1.7	10.96	32.30
	Zn+P ₃₀	0.94	1.9	11.93	40.67
	Zn ₃₀ +P	0.90	1.8	11.43	40.27
	ZnD+P	0.95	2	13.03	45.67
	ZnD ₃₀ +P	0.96	2.1	13.30	50.77
	ZnD+P ₃₀	0.97	2.2	13.83	57.20
L.S.D(0.05)		0.04	0.31	0.68	0.79

added together without BS to reach 14.2, 12.9, 147.3 and 218.0 for roots' zinc concentration, vegetative total weight, and content of zinc in the root and vegetative total weight, respectively while the BS had a significant effect in the same indicators when adding Zn+P with values of 16.2, 13.1, 192.0 and 289.0, respectively.

3.4 Phosphorus and zinc in Soil

It is noticed from Fig. 1 that addition of Zn and ZnD before or after P addition (*i.e.* Zn+P30, Zn30+P, ZnD+P, ZnD30+P and ZnD+P30 treatments) fulfilled the highest values of phosphorus concentration in soil. Despite the insignificant differences between Zn

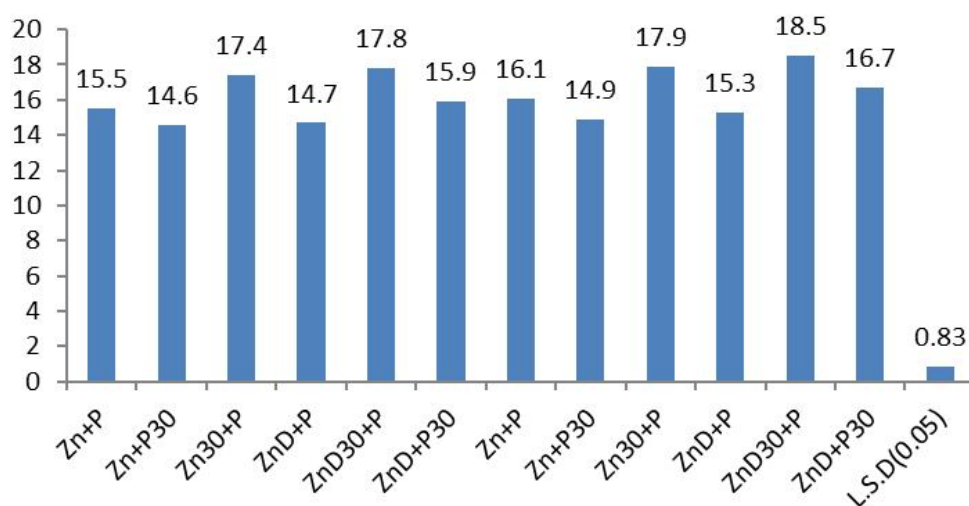


Fig. 1: Effect of experimental treatments on P concentrations in soil

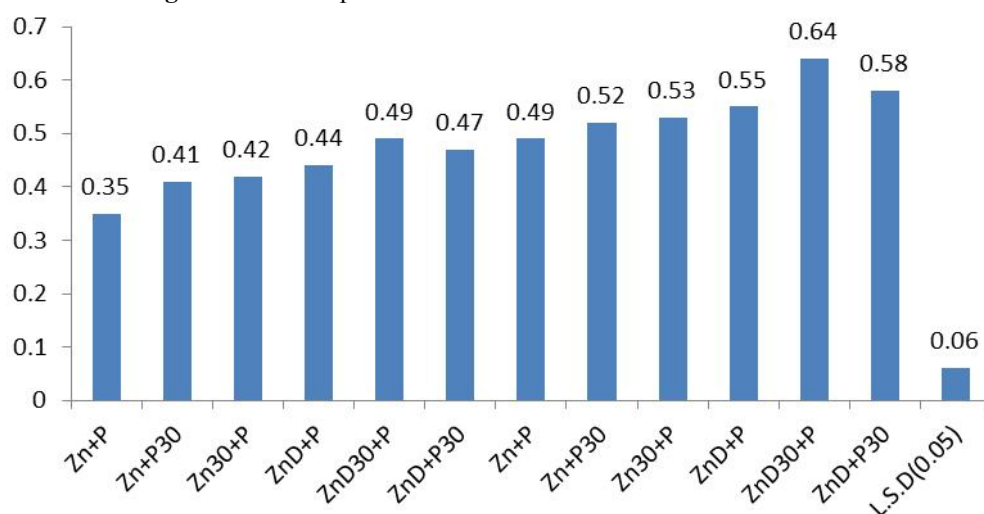


Fig. 2: Effect of experimental treatments on Zn concentrations in soil

sources pre and post addition of P, but the ZnD30+P treatment with BIS had the highest values of P concentration in the soil which reached 18.5 mg P.Kg⁻¹ soil, whereas the least value of P concentration in soil was 15.5 mg P.Kg⁻¹ soil in the Zn+P without BIS treatment which did not differ from soil phosphorus significantly and reached 16.1 mg P.kg⁻¹ soil in the Zn + P without BIS treatment.

As for the concentration of zinc in soil (Fig. 2), it was noticed that the addition of mineral and chelated sources of zinc before or after the addition of phosphorus (*i.e.* Zn+P30, Zn30+P, ZnD+P, ZnD30+P, and ZnD+P30) achieved the highest concentration values of zinc in the soil and although there are no significant differences between the sources of zinc after or before the addition of phosphorus (Zn+P30, Zn30+P, ZnD30+P and ZnD+P30), the Zn30+P treatment with the presence of BIS achieved the highest values in the

concentration of zinc as 0.64 mg Zn.Kg⁻¹ soil and the least value of zinc in the soil was observed in Zn + P treatment without BIS, reached to 0.53 mg Zn.Kg⁻¹, while the BIS had a significant effect when adding zinc with phosphorus (Zn + P) at cultivation compared to the non-BIS treatment.

4. Discussion

It is noticed that best results are associated with ZnD plus BIS treatments compared with mineral zinc treatment. The highest P concentration or content in shoots and roots weight stated at Zn+P30 and ZnD+P with BIS existence because of the increase in P stabilization and decrease in its availability as a result of early addition at the cultivation of phosphorus in calcareous soils that have light alkali pH (7.6), the increase of soil pH leads to decrease in availability and sufficiency of phosphorus to plant especially at sewing stage [Yash *et al.* (1992)].

Table 4: The impact of experimental treatments on phosphorus concentration and content in the vegetative and root total weight.

Inoculation	Fertilizer treatments	Zinc concentration (mg.kg ⁻¹)		Zinc content (µg pot ⁻¹)	
		Root	Shoot	Root	Shoot
Without inoculation	Zn+P	14.2	12.9	147.3	218.0
	Zn+P ₃₀	14.4	13.4	154.7	243.0
	Zn ₃₀ +P	14.5	13.5	170.3	267.0
	ZnD+P	15.8	14.2	190.7	299.0
	ZnD ₃₀ +P	16.7	14.5	220.0	343.7
	ZnD+P ₃₀	16.6	14.4	206.3	318.3
With inoculation	Zn+P	16.2	13.1	192.0	248.0
	Zn+P ₃₀	16.6	13.6	216.7	290.3
	Zn ₃₀ +P	17.8	13.7	220.7	306.0
	ZnD+P	18.2	15.1	250.7	344.3
	ZnD ₃₀ +P	19.1	15.6	270.3	399.7
	ZnD+P ₃₀	18.4	15.4	255.3	377.0
L.S.D(0.05)		0.72	0.66	14.0	26.6

The highest concentration of zinc and its content in the shoot and root total weight achieved when phosphorus was added at cultivation and when it was added to the chelated and mineral zinc after 30 days of cultivation in the presence and absence of BIS. This result depicts the relationship between zinc and phosphorus as an interaction relationship, but in certain cases, there is an opposite relationship and in other cases, there is a stimulation (antagonism) relationship according to crop requirements or the quantity of added phosphate-fertilizer. There is also the effect of adding zinc fertilizer in the absorption of other nutrients such as copper and iron [Al-Sumaidia (2011)].

Bio-inoculation has provided enough phosphorus for the plant, especially in the early start of the growth stage, which has increased the accumulation of nutrients in the plant, due to the various capacities of the organisms associated with a nutrient dissolvent, the production of growth auxins, stimulating the growth of the roots, and then increasing their absorption capacity. These microorganisms can dissolve insoluble forms of nutrients through acidification, chelation and organic acid production [Behera *et al.* (2017)]. Furthermore, plant bio-inoculation has created the best radical system for absorbing water and nutrients compared to non-inoculated plants, which will increase the growth of roots and nutrient absorption through the production of growth auxins and nutrient dissolving. This development in plant

roots has affected the rate of nutrient absorption and transmission to other plant parts [Giehl and von Wiren (2014)]. The addition of chemical fertilizers has undoubtedly increased the concentration of nutrients in the soil, but bio-inoculation has increased the vital availability of both additives and already present nutrients in the soil through solubility, acid production, and phosphatase enzyme activity or chelation [Hussain *et al.* (2015)].

The best results at the presence of chelated in comparison with mineral zinc are attributed to the high adsorption capacity of the later by soil colloids compared to chelated zinc. Furthermore, there is a joint significant correlation between additional mineral zinc and carbonate compared to a chelated form of zinc.

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