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Article

Effect of Zinc-Phosphorus Interaction on Corn Silage Grown on Sandy Soil

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Abstract: This study investigated the response of corn silage to different combinations of zinc (Zn) and phosphorus (P) soil supply when grown in sandy soil. The soil was naturally poor in extractable Zn and rich in plant-available P. The experiment was conducted in outdoor containers. The treatments consisted of soil supply combinations of 3 levels of Zn (0, 5 and 10 mg Zn kg^{-1} of dry soil) and 4 levels of P (0, 12, 36 and 72 mg P_2O_5 kg^{-1} of dry soil). The results showed the absence of a significant effect (at $p \leq 0.05$) of Zn-P interaction on plant growth, plant mineral content or total aerial dry weight at harvest. P application depressed Zn shoot content, and conversely, Zn supply slightly reduced P shoot content. The total aerial dry weight at harvest was not enhanced by P application. However, it was significantly increased by Zn supply of 5 mg·kg^{-1} only for the highest P (72 mg·kg^{-1}) application (at $p \leq 0.05$). This increase was around 15% compared to no Zn soil supply. It was especially linked to kernel dry weight and particularly to pollination rate. For the highest level of P supply, Zn applications significantly enhanced (at $p \leq 0.05$) the kernel dry weight and the pollination rate by 22.1% and 38.4% respectively, compared to no Zn supply.

Keywords: zinc deficiency; phosphorus; corn silage; sandy soil

1. Introduction

Zinc (Zn) deficiency is a common nutritional constraint for crop production, particularly cereals. As is well documented, corn is known to be very sensitive to Zn deficiency stress [1,2]. This deficiency was reported in sandy soils, waterlogged soils and soils with high phosphorus (P) content [3]. It is reported that P fertilization may aggravate Zn deficiency in many crops [1,4,5]. In this regard, a diminishing effect of P fertilization on plant Zn content was reported in corn [6], in wheat [7,8], and in dwarf bean [9]. The Zn-P interaction was explained by numerous reasons such as the dilution of Zn due to the enhancement of plant growth with P, the low translocation of Zn from root to shoot due to the interference with P, and the reduction of Zn availability due to the interaction of Zn with P in soil [1,10]. In a recent study, Ova *et al.* [7] found out that Zn and P interaction in wheat depends on mycorrhiza development, which is reduced under high soil P content. Such reduction minimized the Zn plant absorption. On the other hand, other responses from corn to Zn and P supply have been reported. For example, Brown *et al.* [11] found a mutual depressing effect from Zn and P on corn. Another example is Mallarino and Webb [12], who observed that continuous P fertilization at high rates did not aggravate Zn deficiency in corn. Similarly, a positive relationship between Zn and P content was noted in corn grown on a calcareous soil [5] and on oilseed rape [13].

In the sandy soil of the Loukkos area (Northwestern Morocco), poor in Zn and relatively rich in P, adequate Zn soil supply corrects Zn deficiency and enhances silage yield [14]. Furthermore, it appears that soil P application enhances corn growth even if this soil is naturally rich in P. However, such application seems to intensify the visual Zn deficiency symptoms, exhibited as white areas between the midrib and the margin of leaves. Despite the numerous studies undertaken to explain Zn-P interaction in corn, the magnitude of this interaction in silage yield and its specific mechanism are still not clear. The current work is undertaken to test the interactive effect of differential rates of Zn and P supply on growth, on mineral content, and on biomass production of corn silage grown on sandy soil.

2. Experimental Section

2.1. Experimental Soil

Sandy soil samples were collected from the sandy area of the Loukkos perimeter (34,96° N, 6,21° W, Northwestern Morocco). The soil was air dried, sieved and homogenized. The soil is sandy (87.3% sand, 8% of clay and 5.6% of silt), with a low Di-Ethylene Triamine Penta Acetic acid (DTPA) extractable Zn (0.23 $mg \cdot kg^{-1}$) and a relatively high Olsen P content (38 $mg \cdot kg^{-1}$ of P_2O_5). It is not calcareous and has a pH of 6.1. The other basic soil chemical properties are presented in Table 1.

Table 1. Physicochemical properties of the soil.

Soil Property	
pH (soil: water ratio of 1:5)	6.1
Electrical conductivity ($dS \cdot m^{-1}$) (soil: water ratio of 1:5)	0.08
Cation exchange capacity (meq 100 g^{-1}) (Cobaltihexamine Chloride method)	4.4
Organic matter (%) (Walkley and Black method)	1.06
Olsen P_2O_5 ($mg \cdot kg^{-1}$)	38
Extractable K_2O ($mg \cdot kg^{-1}$) a	73
Extractable MgO ($mg \cdot kg^{-1}$) a	91
Extractable CaO ($mg \cdot kg^{-1}$) a	972
Extractable Zn ($mg \cdot kg^{-1}$) b	0.23
Extractable Cu ($mg \cdot kg^{-1}$) b	0.19
Extractable Mn ($mg \cdot kg^{-1}$) b	47.48
Extractable Fe ($mg \cdot kg^{-1}$) b	22.42

Extractants: a, Ammonium Acetate; b, Diethylene Triamine Penta-Acetic acid (DTPA).

2.2. Experimental Design and Crop Management

Containers (0.42 m length, 0.25 m width and 0.16 m depth) were filled with 20 kg of air dried sandy soil. 5 seeds of maize (*cv.* Panama) were sown in each container. Thinning was done 7 days after emergence to keep one plant per container. The experiment was conducted in outdoor conditions. During the growing season (May to August 2014), the average maximum and minimum temperatures were 33 °C and 17 °C, respectively. Also, no precipitation was recorded during this period. Watering was done whenever required and the soil was irrigated up to its field capacity.

The treatments consisted of different soil supply combinations of 3 levels of Zn (0 or no Zn supply; 5 and 10 mg Zn kg^{-1} of dry soil) and 4 levels of P (0 or no P supply; 12; 36 and 72 mg P_2O_5 kg^{-1} of dry soil). Zn was supplied as a solution of Zn sulfate ($ZnSO_4 \cdot 7H_2O$) and P as a solution of di-ammonium phosphate (DAP). These treatments were split among 3 different times during the growing season: (i) 50% immediately after sowing; (ii) 25% at 4–5 leaf stage; (iii) and 25% at 8–9 leaf stage. The experimental design was a randomized complete block with 4 replications.

The soil was also supplied during the growing season with 371 $kg \cdot ha^{-1}$ of nitrogen (N) as ammonium nitrate, 300 $kg \cdot ha^{-1}$ of K_2O as soluble potassium sulfate, 1.6 $kg \cdot ha^{-1}$ of manganese (Mn) as manganese sulfate, 2.5 $kg \cdot ha^{-1}$ of copper (Cu) as copper sulfate and 2 $kg \cdot ha^{-1}$ of boron (B) as boron sulfate. In order to assure a homogeneous N fertilization for all treatments, an equivalent amount of the difference between the ammonium brought by DAP for the highest P supply and the other P treatments was applied as ammonium sulfate.

Fungal disease (*Helminthosporium*) was controlled by one application of flusilazole at the 7–8 leaf stage. Each container was equipped with a leaching system in order to reuse the leaching solution.

2.3. Measurements

Stem height, stem diameter and leaf area per plant were determined at harvest, which was done approximately at shoot moisture content of 66%. The leaf area was measured using Formula (1) reported by Mokhtarpour *et al.* [15] for corn:

$$\text{Total leaf area per plant} = \sum_{i=1}^{j=n} (L \times W \times 0.75) \tag{1}$$

where L, W, and n are leaf length, leaf greatest width, and last leaf of corn, respectively.

The harvest was done on August, 2014. Plants were separated into stem, leaves and ear. The ear was separated into kernels, husks and cob. These plant's parts were oven dried at 70 °C until constant weight and dry weights were recorded. Also, different kernels yield components were determined: (i) number of kernels per ear; (ii) pollination rate using Formula (2); (iii) and 1000 kernel dry weight.

$$\text{Pollination rate} = \frac{\text{Number of kernels per ear}}{(\text{Number of kernels per ear} + \text{Number of sterilized ovules per ear})} \times 100 \tag{2}$$

In order to determine mineral contents including Zn, P, K, N, Mg, Mn and Cu in shoot and in root at harvest, all fractions of aerial plant's parts and roots were ground to pass through a screen with 0.25 mm openings. To note, the plant roots were rubbed by hand and washed several times with tap water and finally with dionized water to remove soil particles before being ground. 2 g of the ground material, for each treatment's replication, was digested with a tri-acid mixture (nitric, perchloric, and sulfuric acids). Then, the digested material was analyzed for Zn, Mn and Cu using an atomic absorption spectrophotometer (Varian AA 240 Fast Sequential; air acetylene flame, Varian Technologies, Victoria, Australia). Concerning P, N, Mg and K contents, 0.6 g of the ground material was digested with a di-acid mixture (salicylic and sulfuric acids). Then, the digested material was analyzed for P, Mg and K using an inductively coupled plasma spectrophotometer (iCAP 7400 DUO, Thermo Scientific, China) while N content was determined colorimetrically on a Skalar San^{++} autoanalyzer (Skalar Analytical B.V., Breda, Netherlands) according to the Skalar standard method.

A soil sample from each treatment's replication was taken to determine its content in terms of extractable Zn and assimilable P after harvest. It was oven dried at 40 °C and ground to pass a 2 mm sieve. The exchangeable Zn was determined by DTPA extractant method [2] using the atomic absorption spectrophotometer mentioned above. The available P was extracted with Sodium bicarbonate ($NaHCO_3$) [16] and analyzed colorimetrically using an UV-Visible spectrophotometer (Cary 50 Conc, Varian Technologies, Victoria, Australia).

2.4. Statistical Analysis

Experimental data were subjected to analysis of variance (ANOVA) in order to evaluate the significance of the treatments and their interactions effect ($p \leq 0.05$ level). Differences between means of different combinations were compared by Duncan's multiple range test ($p \leq 0.05$ level). All statistical analyses are performed using the SPSS software (Version 17.0, SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Stem Height, Stem Diameter and Leaf Area at Harvest

The stem height and the leaf area were not significantly influenced by Zn-P interaction. The significant effect of Zn application on stem height was observed only for the highest P supply (72 mg·kg^{-1}). At this level of P, Zn supply resulted in a significant increase around 5.8% compared to no Zn supply (Table 2). This significant effect can be explained by the depressing effect of high P soil content in the reduction of Zn availability [1,10]. Many authors reported the role of Zn in stem stretching through its involvement in the metabolism of the Indole 3 Acetic acid as a growth regulator [17]. However, the leaf area did not show a significant increase in terms of Zn application for all P supply levels. On the other hand, a positive response from stem height and leaf area to P application was recorded only at 5 mg·kg^{-1} of Zn. Such a behavior was not understood and is reason for further investigation. At this level of Zn, the highest P supply (72 mg·kg^{-1}) resulted in increases of around 7.08% and 7.4% in stem height and in leaf area respectively compared to no P application. The positive effect of P supply can be explained by the key role of this macro-nutrient in enhancing cell division activity which stimulates growth parameters [18]. Besides, no obvious stem diameter response was observed even though significant effects of Zn-P interaction and P were recorded (Table 2).

Table 2. Effect of zinc and phosphorus supply on stem height, stem diameter and leaf area of corn silage at harvest.

P_2O_5 (mg·kg^{-1})	Zn (mg·kg^{-1})	Stem Height (cm)	Stem Diameter (cm)	Leaf Area (dm²)
0	0	204.66 ± 6.78 ^{c}	2.42 ± 0.06 ^{a,b}	60.10 ± 1.77 ^{b}
	5	205.00 ± 2.96 ^{c}	2.35 ± 0.04 ^{b,c}	61.41 ± 1.22 ^{b}
	10	214.87 ± 4.34 ^{a,b,c}	2.54 ± 0.04 ^{a}	64.89 ± 2.55 ^{a,b}
12	0	210.25 ± 4.47 ^{a,b,c}	2.24 ± 0.07 ^{c}	61.25 ± 0.31 ^{b}
	5	213.00 ± 3.00 ^{a,b,c}	2.42 ± 0.04 ^{a,b}	62.98 ± 0.84 ^{a,b}
	10	212.37 ± 4.43 ^{a,b,c}	2.49 ± 0.07 ^{a,b}	63.30 ± 1.27 ^{a,b}
36	0	213.66 ± 1.25 ^{a,b,c}	2.48 ± 0.06 ^{a,b}	60.70 ± 0.99 ^{b}
	5	218.75 ± 1.9 ^{a,b}	2.48 ± 0.04 ^{a,b}	65.75 ± 2.78 ^{a,b}
	10	219.87 ± 1.85 ^{a}	2.40 ± 0.01 ^{a,b}	62.69 ± 1.43 ^{a,b}
72	0	207.33 ± 2.89 ^{b,c}	2.50 ± 0.05 ^{a,b}	64.41 ± 2.92 ^{a,b}
	5	220.62 ± 2.4 ^{a}	2.54 ± 0.01 ^{a}	67.73 ± 2.03 ^{a}
	10	218.37 ± 4.38 ^{a,b}	2.50 ± 0.02 ^{a,b}	67.94 ± 1.55 ^{a}
P		*	*	*
Zn		*	n.s.	*
P × Zn		n.s.	*	n.s.

Data are the means ± standard error (n = 4). For each measured parameter, means without common letter are significantly different (at $p \leq 0.05$), according to Duncan test. * Significant at 5% probability level; n.s. not significant at 5% probability level.

3.2. Shoot Dry Weight and Partitioning into Ear, Stem, Leaves and Kernels

The total aerial dry weight at harvest, which is equivalent to silage yield, was not influenced by Zn-P interaction. However, it was significantly enhanced by Zn supply only for the highest P

(72 mg·kg^{-1}) application. This increase was around 15% at Zn application of 5 mg·kg^{-1} compared to no Zn soil application (Table 3). This significant response can be explained by the depressing effect of high P soil content in the reduction of Zn availability [1,10]. The beneficial effect of Zn in biomass production was reported in corn [14,19,20], in wheat [21], and in tomato [22]. Such an effect can be explained by the role of Zn in the metabolism of the β-carbonic anhydrase as a crucial enzyme involved in photosynthesis [23]. On the other hand, no significant effect from P supply was recorded. This can be explained by the adequate native Olsen P in soil (38 mg·kg^{-1}). Therefore, P application can be avoided in this kind of soil.

The shoot dry matter partitioning showed that ear and particularly kernel dry weights were significantly enhanced by Zn supply only for the highest P (72 mg·kg^{-1}) application. The increase of kernel dry weight was around 38.43% compared to no Zn soil supply. Furthermore, the biomass production response was particularly related to the kernel dry matter, which represents around 34% of the total shoot dry weight. This result agreed with our previous findings on corn responses to Zn soil supply [14,20] and to Zn foliar spray [19]. Concerning stem and leaves dry matter, they did not exhibit significant responses towards P and Zn supply.

Table 3. Effect of zinc and phosphorus supply on shoot dry weight of corn silage at harvest and its partitioning into stem, leaves, ear and kernels.

P_2O_5 (mg·kg^{-1})	Zn (mg·kg^{-1})	Total Aerial Dry Weight (g·plant^{-1})	Ear Dry Weight (g·plant^{-1})	Stem Dry Weight (g·plant^{-1})	Leaves Dry Weight (g·plant^{-1})	Kernel dry Weight (g·plant^{-1})
0	0	297.23 ± 14.27 ^{a,b}	152.44 ± 8.76 ^{a,b,c}	107.09 ± 11.04 ^{a}	37.70 ± 2.02 ^{a,b}	101.87 ± 10.43 ^{a,b}
	5	305.41 ± 4.11 ^{a,b}	160.94 ± 2.31^{a,b}	106.01 ± 3.46 ^{a}	38.46 ± 1.02 ^{a,b}	112.01 ± 2.77 ^{a}
	10	320.59 ± 2.12 ^{a}	169.45 ± 1.69^{a,b}	111.39 ± 0.98 ^{a}	39.74 ± 0.51 ^{a,b}	116.17 ± 0.94 ^{a}
12	0	296.45 ± 14.73 ^{a,b}	152.41 ± 8.77 ^{a,b,c}	106.32 ± 6.57 ^{a}	37.72 ± 1.54 ^{a,b}	102.44 ± 7.61 ^{a,b}
	5	305.39 ± 11.97 ^{a,b}	171.12 ± 6.79 ^{a}	104.00 ± 3.29 ^{a}	30.26 ± 8.64 ^{b}	117.59 ± 4.28 ^{a}
	10	308.11 ± 13.34 ^{a,b}	163.08 ± 8.51 ^{a,b}	106.83 ± 6.23 ^{a}	38.19 ± 1.06 ^{a,b}	112.33 ± 5.92 ^{a}
36	0	293.91 ± 7.51 ^{a,b}	140.07 ± 11.00 ^{b,c}	115.94 ± 5.94 ^{a}	37.89 ± 0.70 ^{a,b}	89.61 ± 11.90 ^{a,b}
	5	295.58 ± 14.56 ^{a,b}	142.30 ± 13.22 ^{a,b,c}	112.53 ± 4.41 ^{a}	40.73 ± 1.21 ^{a}	95.43 ± 10.31 ^{a,b}
	10	321.48 ± 2.25 ^{a}	167.29 ± 1.31 ^{a,b}	114.35 ± 1.61 ^{a}	39.83 ± 2.07 ^{a,b}	112.99 ± 2.93 ^{a}
72	0	280.51 ± 10.49 ^{b}	124.25 ± 12.44 ^{c}	115.47 ± 8.10 ^{a}	40.78 ± 2.27 ^{a}	76.68 ± 13.82^{b}
	5	325.11 ± 4.73 ^{a}	168.77 ± 3.91 ^{a,b}	114.12 ± 5.34 ^{a}	42.21 ± 1.41 ^{a}	115.65 ± 3.95 ^{a}
	10	297.97 ± 14.63 ^{a,b}	145.73 ± 12.68 ^{a,b,c}	111.07 ± 5.68 ^{a}	41.16 ± 2.14 ^{a}	96.66 ± 13.74 ^{a,b}
P		n.s.	n.s.	n.s.	n.s.	n.s.
Zn		*	*	n.s.	n.s.	*
P × Zn		n.s.	n.s.	n.s.	n.s.	n.s.

Data are the means ± standard error (n = 4). For each measured parameter, means without common letter are significantly different (at $p \leq 0.05$), according to Duncan test. * Significant at 5% probability level; n.s. not significant at 5% probability level.

3.3. Kernels Yield Compounds

As can be seen in Table 4, the response of kernel dry weight was particularly related to the response of the pollination rate. For no Zn supply, high P application (72 mg·kg^{-1}) induced a significant decrease of 21.50% in the pollination rate compared to no P supply. Also, at the highest level of P, Zn applications significantly enhanced the pollination rate, by around 22.14% compared to no Zn supply. Such results showed the depressing effect of high levels of P application without Zn supply on the pollination rate. The crucial role played by Zn in pollination was reported in previous studies conducted in the same sandy soil [14,19,20]. This role is particularly linked to the enhancement of male fertility [24]. The positive response of the pollination rate resulted in an increase in the number of kernels per ear. This latter not only elevated the biomass production but also enhanced the silage quality through its content of kernels. On the other hand, 1000 kernel dry weight did not significantly respond to the studied treatments.

Table 4. Effect of zinc and phosphorus supply on the number of kernels per ear, pollination rate and 1000 kernel dry weight of corn silage at harvest.

P_2O_5 ($mg \cdot kg^{-1}$)	Zn ($mg \cdot kg^{-1}$)	Number of Kernels per Ear	Pollination Rate (%)	1000 Kernels Dry Weight (g)
0	0	576.33 ± 29.31 ^{a,b,c}	91.65 ± 3.62 ^{a,b,c}	195.97 ± 8.70 ^{a,b}
	5	584.00 ± 30.17 ^{a,b,c}	87.35 ± 3.89 ^{a,b,c,d}	192.75 ± 6.40 ^{a,b}
	10	591.20 ± 13.00 ^{a,b}	94.49 ± 0.98 ^{a}	196.88 ± 5.91 ^{a,b}
12	0	537.00 ± 34.88 ^{a,b,c,d}	81.18 ± 5.12 ^{b,c,d,e}	190.68 ± 5.42 ^{a,b}
	5	620.25 ± 15.91 ^{a}	93.31 ± 1.70 ^{a,b}	189.53 ± 4.18 ^{a,b}
	10	580.00 ± 28.64 ^{a,b,c}	89.90 ± 2.80 ^{a,b,c,d}	193.74 ± 4.68 ^{a,b}
36	0	527.33 ± 37.04 ^{b,c,d}	79.45 ± 3.66 ^{c,d,e}	189.31 ± 3.47 ^{a,b}
	5	478.25 ± 39.53 ^{d}	78.50 ± 7.97 ^{d,e}	198.49 ± 6.59 ^{a,b}
	10	555.50 ± 21.33 ^{a,b,c,d}	85.95 ± 3.26 ^{a,b,c,d}	203.75 ± 3.68 ^{a}
72	0	497.66 ± 54.05 ^{c,d}	71.92 ± 3.44 ^{e}	183.80 ± 10.06 ^{b}
	5	620.75 ± 13.66 ^{a}	89.18 ± 3.48 ^{a,b,c,d}	186.20 ± 2.97 ^{a,b}
	10	588.00 ± 25.13 ^{a,b,c}	86.56 ± 1.97 ^{a,b,c,d}	187.63 ± 7.71 ^{a,b}
P		*	*	*
Zn		*	*	n.s.
PX Zn		*	*	n.s.

Data are the means ± standard error ($n = 4$). For each measured parameter, means without common letter are significantly different (at $p \leq 0.05$), according to Duncan test.* Significant at 5% probability level; n.s. not significant at 5% probability level.

3.4. Shoot and Root Mineral Content

As shown in Table 5, Zn shoot content was significantly enhanced by Zn supply and was significantly reduced by P. The highest shoot content, around 10 $mg \cdot kg^{-1}$, was recorded under no P application combined with Zn supply of 10 $mg \cdot kg^{-1}$. The depressed effect of P on Zn shoot content was reported in corn by Takkar *et al.* [6], in rice by Haldar and Mandar [4] and in wheat by Zhu *et al.* [8]. The Zn uptake exhibited the same response trend as Zn shoot content (DATA not shown). Thus, the phenomenon of Zn dilution due to the enhancement of plant growth with P [11] seems not to be the prominent factor in Zn-P interaction in our case study. On the other hand, Zn shoot content was still below the threshold of 22 $mg \cdot kg^{-1}$ required in corn [3] for all treatments. Such a result can be explained by the limited soil volume in container which may highly reduce available soil Zn compared to field condition. Concerning P shoot content, it was significantly enhanced by P supply while it was slightly and significantly reduced by Zn supply. However, all treatments had P shoot content around the requested level for corn production (0.1%–0.5%) [25]. Also, P shoot content did not exceed the phytotoxic threshold of 2% reported by Ova *et al.* [7] even with high P supply (72 mg·kg^{-1}). These results suggest that Zn and P supply induced a mutual depressed effect on each other's shoot content. A similar result was reported by Ova *et al.* [7] in wheat and by Brown *et al.* [11] in corn. Nevertheless, this depressed effect did not significantly affect plant growth and shoot dry matter, but may reduce silage mineral nutrient quality. On the other hand, Zn application reduced Mn shoot content at the highest and the lowest dose of P. It also reduced Cu shoot content at the highest P supply. However, all treatments had required shoot contents in Mn and Cu [26]. The depressed effect of Zn supply on Mn and Cu shoot content was reported with Zn foliar spray in corn silage grown in the same sandy soil [19]. Also, a significant diminishing effect from P application on K shoot content was recorded. Nevertheless, all treatments showed an adequate K shoot content around 0.7% [26], and no K deficiency symptoms has been revealed. In contrast, no significant implication of Zn and P applications on N shoot content was noticed.

In terms of the root mineral content, no significant effect of Zn-P interaction was recorded. The root Zn content was significantly elevated with increasing Zn supply. Further, no Zn accumulation in the root under high P application was recorded. This result is in contrast with the previous finding by Dwivedi *et al.* [27] in corn. Also, the P root content was enhanced by P application and no significant effect from Zn supply was exhibited. The absence of Zn and P accumulation in roots suggested that the mutual depressed effect of Zn and P supply on each other's shoot content was not related to their translocation from root to shoot. Such a result can be explained by the effect of P in limiting plant Zn absorption [10]. In this regard, Ova *et al.* [7] found out that the reduction of wheat's Zn absorption is due to the limited mycorrhiza development under high P soil supply. On the other hand, no significant implication of P and Zn supply in root content of N, K, Mg, Mn and Cu was revealed (Table 6).

3.5. Residual Soil Content on Exchangeable Zn and on Olsen P

After harvest, the residual soil content in exchangeable Zn was significantly elevated with increasing Zn supply. It was, under 5 and 10 mg·kg^{-1}of Zn supply and for different P applications, higher than the threshold of 0.8 mg·kg^{-1} requested for corn production [2]. Thus, the hypothesis of Zn sorption to soil components under high P soil content [28] seems unlikely to occur. On the other hand, the Olsen P soil content increased significantly with increasing P supply while no significant effect from Zn supply was recorded (Table 7).

Table 5. Effect of zinc and phosphorus supply on shoot mineral content of corn silage at harvest.

P_2O_5 (mg kg^{-1})	Zn (mg·kg^{-1})	Shoot Mineral Content						
		Zn (mg·kg^{-1})	P (%)	N (%)	K (%)	Mg (%)	Mn (mg·kg^{-1})	Cu (mg·kg^{-1})
0	0	6.06 ± 0.16 ^{e,f,g}	0.14 ± 0.00 ^{c,d,e}	0.85 ± 0.03 ^{a}	0.74 ± 0.03 ^{a,b}	0.23 ± 0.01 ^{a}	56.06 ± 3.63 ^{a}	1.99 ± 0.10 ^{a,b}
	5	10.04 ± 0.45 ^{a,b}	0.14 ± 0.00 ^{c,d}	0.83 ± 0.02 ^{a}	0.78 ± 0.02 ^{a}	0.21 ± 0.01 ^{a}	48.14 ± 2.59 ^{a,b,c}	2.26 ± 0.13 ^{a}
	10	10.88 ± 1.11 ^{a}	0.12 ± 0.00 ^{e}	0.88 ± 0.03 ^{a}	0.72 ± 0.04 ^{a,b,c}	0.22 ± 0.00 ^{a}	44.03 ± 1.50 ^{b,c}	1.88 ± 0.11 ^{a,b}
12	0	5.59 ± 0.23 ^{g}	0.15 ± 0.00 ^{b,c,d}	0.88 ± 0.04 ^{a}	0.74 ± 0.03 ^{a,b}	0.23 ± 0.01 ^{a}	54.25 ± 0.85 ^{a,b}	1.88 ± 0.09 ^{a,b,c}
	5	8.93 ± 0.56 ^{c}	0.13 ± 0.00 ^{c,d,e}	0.84 ± 0.02 ^{a}	0.68 ± 0.02 ^{a,b,c}	0.24 ± 0.01 ^{a}	46.55 ± 2.84 ^{a,b,c}	1.68 ± 0.15 ^{b,c}
	10	9.73 ± 0.57 ^{a,b}	0.13 ± 0.00 ^{d,e}	0.85 ± 0.03 ^{a}	0.71 ± 0.05 ^{a,b,c}	0.20 ± 0.01 ^{a}	44.02 ± 5.24 ^{b,c}	1.60 ± 0.12 ^{b,c}
36	0	5.88 ± 1.02 ^{f,g}	0.16 ± 0.00 ^{a,b}	0.81 ± 0.03 ^{a}	0.73 ± 0.03 ^{a,b}	0.22 ± 0.02 ^{a}	51.90 ± 2.75 ^{a,b}	2.22 ± 0.12 ^{a}
	5	7.58 ± 0.59 ^{c,d,e}	0.13 ± 0.00 ^{c,d,e}	0.81 ± 0.02 ^{a}	0.64 ± 0.04 ^{b,c}	0.24 ± 0.02 ^{a}	46.24 ± 1.64 ^{a,b,c}	1.93 ± 0.14 ^{a,b}
	10	9.06 ± 0.58 ^{b,c}	0.13 ± 0.01 ^{c,d,e}	0.78 ± 0.04 ^{a}	0.73 ± 0.02 ^{a,b}	0.21 ± 0.01 ^{a}	41.15 ± 2.71 ^{c}	1.82 ± 0.19 ^{a,b}
72	0	4.78 ± 0.29 ^{g}	0.16 ± 0.00 ^{a}	0.85 ± 0.03 ^{a}	0.67 ± 0.02 ^{a,b,c}	0.23 ± 0.01 ^{a}	55.16 ± 2.70 ^{a}	1.85 ± 0.18 ^{a,b}
	5	6.92 ± 0.12 ^{d,e,f}	0.14 ^{b} ± 0.01 ^{c,d}	0.83 ± 0.01 ^{a}	0.61 ± 0.04 ^{c}	0.24 ± 0.00 ^{a}	52.71 ± 4.72 ^{a,b}	1.55 ± 0.10 ^{b,c}
	10	7.95 ± 0.43 ^{c,d}	0.15 ± 0.00 ^{b}	0.80 ± 0.02 ^{a}	0.70 ± 0.03 ^{a,b,c}	0.21 ± 0.02 ^{a}	39.61 ± 3.35 ^{c}	1.45 ± 0.21 ^{c}
P		*	*	n.s.	*	n.s.	n.s.	*
Zn		*	*	n.s.	n.s.	*	*	*
PX Zn		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Data are the means ± standard error ($n = 4$). For each measured parameter, means without common letter are significantly different (at $p \leq 0.05$), according to Duncan test.

* Significant at 5% probability level; n.s. not significant at 5% probability level.

Table 6. Effect of zinc and phosphorus supply on root mineral content of corn silage at harvest.

P_2O_5 (mg·kg^{-1})	Zn (mg·kg^{-1})	Root Mineral Content						
		Zn (mg·kg^{-1})	P (%)	N (%)	K (%)	Mg (%)	Mn (mg·kg^{-1})	Cu (mg·kg^{-1})
0	0	3.52 ± 0.87 ^{c,d}	0.07 ± 0.00 ^{a,b}	0.39 ± 0.00 ^{a}	0.17 ± 0.05 ^{a}	0.12 ± 0.00 ^{b}	21.04 ± 2.98 ^{a}	1.94 ± 0.38 ^{a,b}
	5	5.24 ± 0.53 ^{a,b,c}	0.07 ± 0.00 ^{a,b}	0.48 ± 0.02 ^{a}	0.13 ± 0.01 ^{a}	0.13 ± 0.00 ^{a,b}	23.59 ± 1.77 ^{a}	2.38 ± 0.15 ^{a,b}
	10	6.96 ± 0.76 ^{a}	0.08 ± 0.00 ^{a,b}	0.48 ± 0.03 ^{a}	0.20 ± 0.03 ^{a}	0.16 ± 0.02 ^{a}	26.23 ± 2.33 ^{a}	2.14 ± 0.43 ^{a,b}
12	0	3.33 ± 0.63 ^{d}	0.08 ± 0.00 ^{a,b}	0.37 ± 0.02 ^{a}	0.17 ± 0.04 ^{a}	0.12 ± 0.00 ^{b}	23.74 ± 3.00 ^{a}	1.61 ± 0.46 ^{a,b}
	5	5.71 ± 1.21 ^{a,b}	0.06 ± 0.00 ^{b}	0.44 ± 0.00 ^{a}	0.16 ± 0.03 ^{a}	0.12 ± 0.00 ^{b}	23.35 ± 3.38 ^{a}	1.86 ± 0.44 ^{a,b}
	10	5.80 ± 0.35 ^{a,b}	0.07 ± 0.00 ^{a,b}	0.47 ± 0.05 ^{a}	0.17 ± 0.02 ^{a}	0.12 ± 0.00 ^{b}	24.46 ± 2.25 ^{a}	1.69 ± 0.40 ^{a,b}
36	0	3.87 ± 0.47 ^{c,d}	0.08 ± 0.00 ^{a,b}	0.38 ± 0.03 ^{a}	0.13 ± 0.02 ^{a}	0.12 ± 0.00 ^{b}	24.43 ± 1.21 ^{a}	2.58 ± 0.49 ^{a}
	5	4.87 ± 0.35 ^{b,c,d}	0.08 ± 0.00 ^{a}	0.41 ± 0.03 ^{a}	0.18 ± 0.02 ^{a}	0.13 ± 0.01 ^{a,b}	21.84 ± 1.98 ^{a}	1.79 ± 0.27 ^{a,b}
	10	5.86 ± 0.44 ^{a,b}	0.08 ± 0.00 ^{a,b}	0.42 ± 0.01 ^{a}	0.15 ± 0.01 ^{a}	0.13 ± 0.01 ^{a,b}	20.93 ± 2.23 ^{a}	1.82 ± 0.40 ^{a,b}
72	0	4.14 ± 0.42 ^{b,c}	0.09 ± 0.01 ^{a}	0.48 ± 0.06 ^{a}	0.19 ± 0.02 ^{a}	0.13 ± 0.00 ^{a,b}	25.65 ± 1.76 ^{a}	2.37 ± 0.40 ^{a,b}
	5	4.99 ± 0.22 ^{b,c,d}	0.08 ± 0.00 ^{b}	0.39 ± 0.02 ^{a}	0.22 ± 0.03 ^{a}	0.13 ± 0.00 ^{b}	22.49 ± 1.15 ^{a}	2.23 ± 0.65 ^{a,b}
	10	6.78 ± 0.74 ^{a}	0.09 ± 0.00 ^{a}	0.44 ± 0.06 ^{a}	0.18 ± 0.03 ^{a}	0.13 ± 0.00 ^{a,b}	25.04 ± 2.35 ^{a}	1.55 ± 0.19 ^{b}
P		n.s.	*	n.s.	n.s.	n.s.	n.s.	n.s.
Zn		*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
PX Zn		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Data are the means ± standard error ($n = 4$). For each measured parameter, means without common letter are significantly different (at $p \leq 0.05$), according to Duncan test.

* Significant at 5% probability level; n.s. not significant at 5% probability level.

Table 7. Effect of zinc and phosphorus supply on residual soil content of exchangeable zinc (DTPA Extraction) and assimilable phosphorus (Olsen) after harvest.

P_2O_5 (mg·kg^{-1})	Zn (mg·kg^{-1})	Exchangeable Zn (mg·kg^{-1})	Olsen P (mg·kg^{-1})
0	0	0.35 ± 0.01 ^{e}	52.50 ± 6.35 ^{b}
	5	1.76 ± 0.05 ^{d}	46.75 ± 0.47 ^{b}
	10	3.62 ± 0.28 ^{b,c}	49.50 ± 2.25 ^{b}
12	0	0.38 ± 0.02 ^{e}	51.00 ± 1.35 ^{b}
	5	1.80 ± 0.13 ^{d}	54.75 ± 3.19 ^{b}
	10	3.15 ± 0.30 ^{c}	51.50 ± 1.93 ^{b}
36	0	0.36 ± 0.03 ^{e}	60.00 ± 6.14 ^{b}
	5	2.13 ± 0.16 ^{d}	61.00 ± 2.38 ^{b}
	10	3.78 ± 0.11 ^{b}	58.00 ± 2.04 ^{b}
72	0	0.41 ± 0.07 ^{e}	86.50 ± 13.62 ^{a}
	5	1.97 ± 0.12 ^{d}	81.00 ± 9.78 ^{a}
	10	4.52 ± 0.39 ^{a}	79.00 ± 4.88 ^{a}
P		*	*
Zn		*	n.s.
P × Zn		n.s.	n.s.

Data are the means ± standard error ($n = 4$). For each measured parameter, means without common letter are significantly different (at $p \leq 0.05$), according to Duncan test. * Significant at 5% probability level; n.s. not significant at 5% probability level.

4. Conclusions

The results of this study have shown the absence of a significant effect (at $p \leq 0.05$) from Zn-P interaction on corn grown in sandy soil. However, a mutual depressive effect of Zn and P applications on each other's shoot content was recorded. The diminishing effect of high P supply on Zn shoot content was more pronounced than that induced by Zn on P shoot content. The depressive effect of P supply on Zn shoot content does not seem to be related either to the hypothesis of low translocation of Zn from root to shoot nor to the hypothesis of Zn sorption to soil components under high P soil content. The biomass production of corn silage was significantly enhanced by Zn supply of 5 mg·kg^{-1} only for the highest P application (72 mg·kg^{-1}) (at $p \leq 0.05$). However, P supply did not show any additional benefit in terms of biomass production.

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Author Contributions

The experiment was designed by Abdelhadi Aït Houssa and was performed by Saad Drissi. The work and the data interpretation were supervised by Mohamed Benbella and Ahmed Bamouh. The

plant and soil mineral analysis were supervised and financed by Jean-Marie Coquant. The manuscript preparation was jointly done by Saad Drissi and Abdelhadi Aït Houssa.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Alloway, J.B. *Zinc in Soils and Crop Nutrition*, 2nd ed.; International Zinc Association Communications; IZA Publications: Brussel, Belgium, 2008. Available online: http://www.zinc.org/general/Zinc_in_Soils_and_Crop_Nutrition_ALLOWAY.pdf (accessed on 12 May 2015).
2. Lindsay, W.L.; Norvell, W.A. Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci. Soc. Am. J.* **1978**, *42*, 421–428.
3. Bhupinder, S.; Senthil, K.A.N.; Singh, B.K.; Usha, K. Improving zinc efficiency of cereals under zinc deficiency. *Curr. Sci.* **2005**, *88*, 36–44.
4. Haldar, M.; Mandal, L.N. Effect of phosphorus and zinc on the growth and phosphorus, zinc, copper, iron and manganese nutrition of rice. *Plant Soil* **1981**, *59*, 415–425.
5. Orabi, A.A.; Mashadi, H.; Abdallah, A.; Morsy, M. Effect of zinc and phosphorus on the grain yield of corn (*Zea mays* L.) grown on a calcareous soil. *Plant Soil* **1981**, *63*, 291–294.
6. Takkar, P.N.; Mann, M.S.; Bansal, R.L.; Randhawa, N.S.; Singh, H. Yield and uptake response of corn to zinc, as influenced by phosphorus fertilization. *Agron. J.* **1975**, *68*, 942–946.
7. Ova, E.A.; Kutman, U.B.; Ozturk, L.; Cakmak, I. High phosphorus supply reduced zinc concentration of wheat in native soil but not in autoclaved soil or nutrient solution. *Plant Soil* **2015**, doi:10.1007/s11104-015-2483-8.
8. Zhu, Y.G.; Smith, S.E.; Smith, F.A. Zinc (Zn)-phosphorus (P) interactions in two cultivars of spring wheat (*Triticum aestivum* L.) differing in P uptake efficiency. *Ann. Bot.* **2001**, *88*, 941–945.
9. Gianquinto, G.; Abu-Rayyan, A.; Tola, L.D.; Piccotino, D.; Pezzarossa, B. Interaction effects of phosphorus and zinc on photosynthesis, growth and yield of dwarf bean grown in two environments. *Plant Soil* **2000**, *220*, 219–228.
10. Pasricha, N.S.; Baddesha, H.S.; Aulakh, M.S.; Nayyar, V.K. The zinc quantity-intensity relationships in four different soils as influenced by phosphorus. *Soil Sci*. **1987**, *143*, 1–4.
11. Brown, A.L.; Krantz, B.A.; Eddings, J.L. Zinc-phosphorus interactions as measured by plant response and soil analysis. *Soil Sci*. **1970**, *110*, 415–420.
12. Mallarino, A.P.; Webb, J.R. Long-term evaluation of Phosphorus and Zinc interactions in corn. *J. Prod. Agric*. **1995**, *8*, 52–55.
13. Lu, Z.G.; Grewal, H.S.; Graham, R.D. Dry matter production and uptake of zinc and phosphorus in two oilseed rape genotypes under differential rates of zinc and phosphorus supply. *J. Plant Nutr.* **1998**, *21*, 25–38.
14. Drissi, S.; Aït Houssa, A.; Bamouh, A.; Benbella, M. Response of corn silage (*Zea mays* L.) to zinc fertilization on a sandy soil under field and outdoor container conditions. *J. Saudi Soc. Agric. Sci.* **2015**, doi:10.1016/j.jssas.2015.05.002.

15. Mokhtarpour, H.; The, C.B.S.; Saleh, G.; Selamat, A.B.; Asadi, M.E.; Kamkar, B. Non destructive estimation of maize leaf area, fresh weight, and dry weight using leaf length and leaf width. *Commun. Biometr. Crop Sci.* **2010**, *5*, 19–26.
16. Olsen, S.R.; Cole, C.V.; Watanabe, F.S.; Dean, L.A. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Agriculture, United States department of agriculture in cooperation with the Colorado agricultural experiment station. *Circular* **1954**, *939*, 1–19.
17. Cakmak, I.; Marshner, H.; Bangerth, F. Effect of zinc nutritional status on growth, protein metabolism and levels of indole 3 acetique acid and other phytohormones in bean (*Phaseolus vulgaris*). *J. Exp. Bot.* **1988**, *40*, 405–412.
18. Balemi T.; Negisho K. Management of soil phosphorus and plant adaptation mechanisms to phosphorus stress for sustainable crop production: A review. *J. Soil Sci. Plant Nutr*. **2012**, *12*, 547–561.
19. Drissi, S.; Aït Houssa, A.; Bamouh, A.; Benbella, M. Corn silage (*Zea mays* L.) response to zinc foliar spray concentration when grown on sandy soil. *J. Agric. Sci.* **2015**, *7*, 68–79.
20. Drissi, S.; Aït Houssa, A.; Bamouh, A.; Daoudi, E.; Benbella, M. Efficacy of dairy cattle slurry in preventing Zinc deficiency of a silage corn (*Zea mays* L.) grown on a sandy soil. *J. Agric. Sci.* **2015**, *7*, 56–66.
21. Haslett, B.S.; Reid, R.J.; Rengel, Z. Zinc mobility in wheat: Uptake and distribution of zinc applied to leaves or roots. *Ann. Bot.* **2001**, *87*, 379–386.
22. Kaya, C.; Higgs, D. Response of tomao (*Lycopersicon esculentum* L.) cultivars to foliar application of zinc when grown in sand culture at low zinc. *Sci. Hortic*. **2002**, *93*, 53–64.
23. Sasaki, H.; Hirose, T.; Watanabe, Y.; Ohsugi, R. Carbonic anhydrase activity and CO_2 Transfer resistance in Zn deficient rice leaves. *Plant Physiol*. **1998**, *118*, 929–934.
24. Sharma, P.N.; Chatterjee, A.S.C.; Saharma, C.P. zinc deficiency and pollen fertility in maize (*Zea mays*). *Plant Soil* **1990**, *124*, 221–225.
25. Arnon, I. *Mineral Nutrition of Maize*; International Potash Institute: Bern, Germany, 1975. Available online: http://www.ipipotash.org/udocs/mineral_nutrition_of_maize.pdf (accessed on 13 March 2015).
26. Rashid, A.; Ryan, J. Micronutrient constraints to crop production in the Near East. Potential significance and management strategies. In *Micronutrient Deficiencies in Global Crop Production*; Alloway, V.B., Ed.; Springer: Amsterdam, The Netherlands, 2008; pp. 149–180.
27. Dwivedi, R.S.; Randhawa, N.S.; Bansal, R.L. Phosphorus-zinc interaction I. Sites of immobilization of zinc in maize at high level of phosphorus. *Plant Soil* **1975**, *43*, 639–648.
28. Loneragan, J.F.; Webb, M.M. Interactions between zinc and other nutrients affecting the growth of plants. In *Zinc in Soils and Plants*, *Developments in Plant and Soil Sciences*; Robson, A.D., Ed.; Springer: Amsterdam, The Netherlands, 1993; Volume 55, pp. 119–134.