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BIOFORTIFICATION OF MAIZE WITH ZINC

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Abstract

The article presents the experimental results obtained in the greenhouse to demonstrate the efficacy of administering zinc to the soil, plant, and seed in order to reduce the incidence of zinc deficiency in maize. For the experimentation in vegetation pots, a late hybrid FAO 430 and an organo-mineral material constituted from topsoil originating from a soil of the calcaric chernozem type, characteristic of the south-eastern region of Romanian Plain which received different treatments with $CaCO_3$, KH_2PO_4 and $ZnSO_4{\cdot}7H_2O$, were used. At 3 to10 days intervals, phenological observations were made on maize plants, and at the end of the experiment, soil and plant samples were collected and analysed in the laboratory. According to obtained data, the most effective method for biofortification of plants with zinc, among the three methods of administration, was the application of zinc to seed. According to an analysis of the absorption of nutritional elements by maize plants, it can be stated that the administration of the microelement both in soil and on seed are optimal methods for biofortifying maize plant with zinc, with practically identical performance.

Key words: *zinc, maize, biofortification, soil, greenhouse.*

INTRODUCTION

Zn deficiency in maize (*Zea mays*) occurs during the well-developed 4-7 leaves phenophase. The phenomenon is typically observed in plants grown in soils with a neutral-alkaline reaction, which are well and extremely well supplied with P but have low Zn levels. Zn deficiency is manifested by reduced plant height, short internodes and a thick appearance, yellowing of young leaves and a parchment paper appearance in the form of whitish bands present along the length of the leaf, parallel to the midrib, when the plants are in an advanced stage of deficiency (Lăcătușu, 2016).

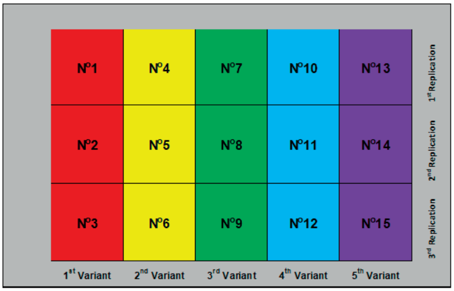
Lately, to reduce the incidence of this phenomenon, in specialized literature the notion of biofortification is used, which involves a process of increasing the contents of micronutrients, in the basic crops consumed on a large scale, through conventional breeding techniques, agronomic practices or genetic modifications (Lividini et al., 2017). Agronomic biofortification is an effective and practical method that provides a quick solution to the problem of maintaining sufficient amount of available Zn in the soil, maximizing plant uptake and adequate transport of Zn to the grain (Cakmak, 2008). In this study, the methods of biofortification (increasing Zn content) in maize plants suffering from this deficiency are presented. In agricultural practice, there are three methods to reduce the occurrence of zinc deficiency or to biofortify plants susceptible to this phenomenon with zinc: (I) application of zinc to the soil; (II) application of zinc to the plant; and (III) application of zinc to the seed prior to its introduction into the soil.

From the research carried out so far, the specialized literature does not present conclusive results regarding the maximum effectiveness brought by one or another of the three stated methods. Therefore, Borlan et al. (1994), recommends with priority the repeated administration of Zn on the plant, at intervals of 4-6 days, with solutions of 0.25-0.40% $ZnSO_4{\cdot}7H_2O$, when the first morphological symptoms of Zn deficiency manifestation appear. It is recommended to apply Zn in the soil, once every 4-5 years in different doses from 4 to 15 kg Zn/ha, depending on the natural supply level of the soil and the nature of the plant to be cultivated (Alloway, 2008; Graham et al., 1992). Zn application on the seed is also practiced. Farooq et al. (2012), carried out a synthesis study regarding the application of microelements (Zn, B, Mo, Mn, Cu, Co) on the seed of different plants (wheat, rice, oats, beans, maize, sunflower, etc.). Regarding Zn, the effect of the treatment on bean, beetroot and maize seeds is highlighted. To these, Harris et al. (2007), obtained a 27% increase in production in plants grown from Zn treated seed compared to the production obtained in untreated plants. Likewise, a 19% increase in production was obtained for chickpeas grown from seeds treated with Zn (Harris et al., 2008).

In light of the fact that no study was found that compared the three methods of applying Zn under identical experimental conditions, we conducted an experiment in which we compared the development of maize plants during the first part of the vegetation period, depending on the method of application, implying the biofortification of maize with Zn.

MATERIALS AND METHODS

The experiment was carried out under greenhouse conditions, and a 12 hours light and 12 hours darkness schedule was maintained throughout the experiment. A constant temperature of 22.3 ± 2.0°C was maintained during the light period, an average luminosity of 4949 ± 1100 lux and an air humidity value of RH equal to 30.6 ± 2.4%. Experiments were carried out with a soil material (0-20 cm) from the surface horizon, the calcareous chernozem characteristic of the South-East Romanian Plain with the following characteristics: pH H_2O (1: 2.5): 7.83; $CaCO_3$: 0.7%; organic matter: 3.50%; N: 0.169%; N-NO_3: 18 mg·kg^{-1}; P_{AL}: 34 mg·kg^{-1}; K_{AL}: 177 mg·kg^{-1}; Zn: 60 mg·kg^{-1}; Cu: 27 mg·kg^{-1}; Mn: 686 mg·kg^{-1}; Co: 8 mg·kg^{-1}; Ni: 31 mg·kg^{-1}; Cr: 31 mg·kg^{-1}; Cd: 0.17 mg·kg^{-1}; Pb: 13 mg·kg^{-1}; Fe: 2.43%. The soil material was loaded into 15 vegetation pots, with 6.0 kg each, and the treatments were carried out according to the data in Figure 1. 4 seeds of late hybrid FAO 430/pot were seeded. During the growing season, soil humidity was maintained at 70% water capacity of the soil in the pot. Phenological observations were made, following the height of the plants at time intervals between 3 and 10 days. Maize plant samples were collected at two times: 44 days after seeding, two plants (aerial part); 58 days after seeding, when the experiment ended, the other two plants (root and aerial part).



Test plant: Maize
Hybrid: FAO 430
Experimental design: **Monofactorial experiment**
Design method: **Pots in greenhouse**
Numer of replications: **3**
Number of variants: **control + 4**

🟥	**1st Variant**	-	Unfertilized martor
🟨	**2nd Variant**	-	P$^{1)}$ 125 mg kg^{-1} + $CaCO_3$ 125 mg kg^{-1}
In soil	**3rd Variant**	-	P$^{1)}$ 125 mg kg^{-1} + $CaCO_3$ 125 mg kg^{-1} + Zn$^{2)}$ 122 mg kg^{-1}
On seed	**4th Variant**	-	P$^{1)}$ 125 mg kg^{-1} + $CaCO_3$ 125 mg kg^{-1} + Zn$^{2)}$ 122 mg kg^{-1}
On plant	**5th Variant**	-	P$^{1)}$ 125 mg kg^{-1} + $CaCO_3$ 125 mg kg^{-1} + Zn$^{2)}$ 122 mg kg^{-1}

$^{1)}$ from KH_2PO_4
$^{2)}$ from $ZnSO_4$ $7H_2O$

Figure 1. The treatments performed on the soil in the vegetation pots

Along with these, soil samples were collected from the entire depth of the pot.

For the soil samples, the following analyzes were performed: pH H_2O (1: 2.5), potentiometric measurements, using a combined glass - calomel electrode; the $CaCO_3$ content, using the volumetric gas method (Scheibler); N content, using Kjeldahl method; NO_3^- content using potentiometric measurements with ion selectiv electrode; the mobile content of P and K were determined by spectrophotometry, respectively flame spectrometry, using extraction with ammonium acetate-lactate solution at pH 3.7, Egnèr-Riehm-Domingo method. The contents of trace elements (Zn, Cu, Fe, Mn), mobile forms, were determined in CH_3COONH_4-EDTA at pH 7.0 (Lăcătuşu et al., 1987) and total forms (Zn, Cu, Fe, Mn), were passed into solution with a mixture of concentrated mineral acids (HNO_3, $HClO_4$, HCl) and hydrogen peroxide. Measurements were performed by atomic absorption spectrophotometry in an air-acetylene flame.

The collected plant material samples (root and aerial part) were analyzed from the point of view of the chemical composition of macro- and microelements. N was determined according to the Kjeldahl method, P was determined by spectrophotometry, K and Ca by flame photometry and Mg, Zn, Cu, Fe and Mn were determined by atomic absorption spectrophotometry in the clorhydric solution obtained after solubilization of ash plant at a temperature of 450°C.

All methods used are technical procedures certified in the ISO and STAS systems. The analytical data obtained were statistically calculated, using ANOVA SPSS 14, Duncan's test.

RESULTS AND DISCUSSIONS

The evolution of average height of maize plants during the vegetation period

During the 58 days that the vegetation period lasted, the plants reached an average height between 59.7 cm in the control variant and 66.3 cm in the variant in which Zn was applied on the seed (Table 1).

Table 1. Average height of maize plants (cm) during the vegetation period

Measurement date / Variant	10 days after seeding	17 days after seeding	23 days after seeding	29 days after seeding	38 days after seeding	48 days after seeding	58 days after seeding
Control	10.6	31.3	43.3 a	47.6 a	50.0 a	56.7 a	59.7 a
0 Zn	10.7	32.0	45.0 ab	47.0 ab	55.0 b	61.7 c	64.0 b
Zn in soil	11.0	31.3	46.3 bc	49.0 ab	57.1 cd	64.7 d	66.1 b
Zn on seed	11.3	33.7	48.0 c	49.7 ab	58.0 c	62.7 c	66.3 b
Zn on plant	10.7	31.7	46.7 bc	48.7 b	55.2 bc	59.7 b	61.3 a

It was observed that the maximum effect of Zn application occurred when the microelement was applied on the seed, compared on the soil application variant. The heights of the plants in these two variants were comparable, as shown by the data in Table 1, Figures 2 and 3 in decreasing order of the plant height, depending on the place where the microelement was applied.

Next is the variant with Zn applied to the plant through foliar application with $ZnSO_4 \cdot 7H_2O$ solution, applied three times at one-day intervals. At each size measurement, the lowest values were recorded in control plants and then in those that did not receive Zn, but received P. The separation of the size of the plants according to the method of Zn application, was evident and statistically significantly ensured after 23 days after seeding, when the plants had developed 3-4 pairs of leaves.

Figure 2. 44 days after seeding - Four plants per pot. MT1 - control; 4 - soil without Zn addition; 9 - Zn applied to the soil; 10 - Zn applied to the seed; 15 - Zn applied to the plant

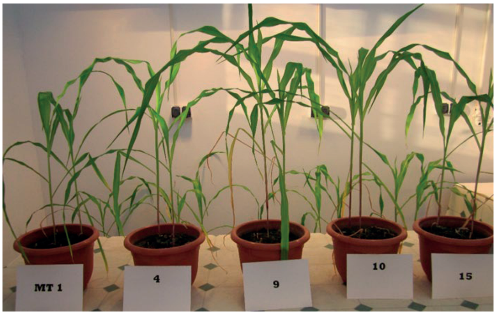


Figure 3. 44 days after seeding - two plants per pot. MT1 - control; 4 - soil without Zn addition; 9 - Zn applied to the soil; 10 - Zn applied to the seed; 15 - Zn applied to the plant

The statistical calculation of all mass values, both green and dry (Table 2) clearly demonstrates the beneficial effect of applying the micronutrient to the soil and the seed, as compared to its application to the plant.

Table 2. Green and dry mass (g) obtained in the experimental variants

Variant	Green mass		Dry mass	
	aerial part	root	aerial part	root
Control	25.0 a	1.1 a	4.0 a	0.8 a
0 Zn	38.7 b	2.7 ab	5.7 a	1.6 ab
Zn in soil	56.0 c	4.3 c	9.0 b	2.5 c
Zn on seed	52.3 c	3.9 c	9.0 b	2.3 c
Zn on plant	34.0 ab	2.7 ab	5.7 a	1.8 ab

The first morphological symptoms of Zn deficiency appeared in the last part of the vegetation period, in the plants to which no Zn was applied (Figure 4) and prior to the application of Zn to the plant (Figure 5).

The addition of zinc to the soil resulted in a greater microelement distribution area and a more uniformly distributed plant nutrient source.

Figure 4. 26 days after seeding - Morphological symptoms of deficiency occurrence in plants in pots without Zn application

Figure 5. 26 days after seeding - Early deficiency symptoms in plants treated with Zn on plant

In an experiment carried out in the greenhouse, Vasconcelos et al. (2011), obtained a 5% increase in the development of maize plants from the variants in which Zn was applied in the soil compared to its application on the plant.

The effect of applied treatments on some agrochemical properties of the soil from the vegetation pots

Following the cessation of plant growth, agrochemical analyzes were conducted on soil samples collected from vegetation pots. Tables 3 and 4 present analytical data that have been statistically calculated.

After $CaCO_3$ application, soil pH increased by 0.32 units, from 7.97 in the control sample to 8.29 in the sample where zinc was applied on plant. But the reaction domain was the same, low-alkaline. In parallel, the $CaCO_3$ content also increased by 0.77%, from 0.73% in the control sample to 1.50 in the sample where zinc was applied on plant. The different $CaCO_3$ concentrations must be attributed to the differential degradation of the applied $CaCO_3$ based on the size of the plant's development. $CaCO_3$ dissolution was most intense in the variants where Zn was applied to the soil, where the plants grew best.

Total N content is relatively constant in all variants with a decrease of up to 0.022% in the variant where Zn was applied on plant. The non-application of any amount of nitrogen, on a medium natural background of assurance with this essential macroelement, contributed to the slight fluctuation of the total and mobile content between the variants.

Table 3. Reaction, content of $CaCO_3$ and macroelements in the soil material present of the vegetation pots at the end of the experiment

Variant	pH_{H2O}	$CaCO_3$	N_t	$N\text{-}NO_3$	P_{AL}	K_{AL}
		%		$mg{\cdot}kg^{-1}$		
Control	7.97 a	0.73 a	0.176 a	9.13 b	32.7 a	192 a
0 Zn	8.13 ab	1.43 a	0.157 a	7.10 ab	59.3 c	255 b
Zn in soil	8.15 ab	1.37 a	0.162 a	6.43 a	54.0 bc	251 b
Zn on seed	8.20 b	1.43 a	0.153 a	5.93 a	48.3 b	241 b
Zn on plant	8.29 b	1.50 a	0.152 a	6.73 ab	49.7 bc	252 c

Table 4. The total and mobile content of microelements ($mg{\cdot}kg^{-1}$) in the soil material present in the vegetation pots at the end of the experiment

Variant	Zn		Cu		Fe		Mn	
	total	mobile	total	mobile	total	mobile	total	mobile
Control	65.1 b	1.47 b	27.7 b	3.13 b	2.65 c	8.33 b	717 b	8.33 b
0 Zn	60.8 a	1.00 a	25.8 a	2.47 a	2.54 c	3.23 a	673 ab	2.27 a
Zn in soil	62.8 ab	2.73 c	25.5 a	2.47 a	2.56 c	3.23 a	705 ab	2.30 a
Zn on seed	60.5 a	1.10 a	25.7 a	2.40 a	2.41 b	2.47 a	652 ab	1.80 a
Zn on plant	61.3 a	1.10 a	26.4 ab	2.47 a	2.27 a	3.00 a	636 a	2.10 a

On the contrary, the application of P in the soil material of the vegetation pots led to obtaining, after ending the experiment, a high content of mobile P. Even under these conditions a differentiation of up to 11 $mg{\cdot}kg^{-1}$ was obtained between the variant that did not receive Zn and the one in which Zn was applied on the seed. The situation is similar regard to K mobile, whose content was also in the high range of supply with a difference of 14 $mg{\cdot}kg^{-1}$ between the variant with zero zinc applied and the variant where zinc was applied on the seed.

Also interesting are the values of microelements contents, both total and mobile, which without exception are higher, in the control variant than in the variants treated according to the presented scheme. The fact that the lowest concentrations of these elements were found in the variant in which Zn was applied to the seed, where the maximum green and dry plant masses were obtained, indicates that the plants absorbed these elements more efficiently.

The evolution of the chemical composition of maize plants according to the method of Zn application

The results of the statistical processing of the chemical analyzes performed regarding the content of macro- and microelements in the aerial part and the root part of the plants are presented in Tables 5-8.

Although the control plants, collected at 44 days after seeding, had a higher N content than the plants grown with Zn intake, the differences were not statistically ensured. However, in the plants collected at 58 days after seeding, the trend was maintained, even at lower content values, being this time statistically ensured. Considering also the values of the N content in the roots, where the highest concentration was in the roots from the control variant, it can be stated that the impact with P and Zn in the other variants contributed to the reduction of N absorption both in the roots and in the aerial part of plants.

In the case of P, the phenomenon was the opposite, the maximum absorption of the macroelement occurred in the plants of the variants treated with P and Zn, both in the roots and in the aerial part. Of course, the amount of 125 mg P applied per kg of soil material in the pot also contributed to this phenomenon. It is observed (Tables 5 and 6) that only the differences from plants collected at 44 days after seeding are statistically ensured.

And in the case of K, higher contents were registered in the plants of the variants treated with P and Zn both in the aerial part, at the two harvests (Table 5) and in the roots (Table 6). The higher mobility of K led to the more intense uptake in the aerial part of the plant and especially in younger plants.

Table 5. Macroelements content (%) of maize plants (aerial part)

Collect date	Variant	N	P	K	Ca	Mg
44 days after seeding	Control	1.92 a	0.24 a	3.19 a	0.73 b	0.57 b
	0 Zn	1.61 a	0.33 b	5.60 b	0.31 a	0.28 a
	Zn in soil	1.40 a	0.29 b	5.39 b	0.31 a	0.28 a
	Zn on seed	1.72 a	0.30 b	4.22 ab	0.33 a	0.27 a
	Zn on plant	1.57 a	0.32 b	4.87 b	0.34 a	0.30 a
58 days after seeding	Control	1.73 b	0.16 a	2.34 a	0.59 b	0.48 b
	0 Zn	1.16 a	0.24 a	3.58 c	0.26 a	0.22 a
	Zn in soil	0.93 a	0.24 a	3.27 bc	0.22 a	0.18 a
	Zn on seed	0.95 a	0.22 a	3.06 b	0.22 a	0.20 a
	Zn on plant	1.11 a	0.24 a	3.39 bc	0.24 a	0.20 a

The two macroelements Ca and Mg analyzed had, as in the case of N, a more intense accumulation in the control plants, both in the aerial part and in the roots. The content differences being statistically ensured, with one exception, the Mg content in the roots (Table 6).

Table 6. Macroelements content (%) of maize plants (root part)

Collect date	Variant	N	P	K	Ca	Mg
58 days after seeding	Control	1.32 b	0.19 a	0.51 a	0.94 c	0.27 a
	0 Zn	1.08 ab	0.21 a	1.94 b	0.65 a	0.25 a
	Zn in soil	0.89 a	0.20 a	2.33 c	0.64 a	0.19 a
	Zn on seed	0.90 a	0.19 a	2.32 c	0.85 bc	0.19 a
	Zn on plant	1.19 b	0.22 a	1.87 b	0.73 ab	0.21 a

Regarding the content of microelements (Tables 7 and 8), differences were observed for Zn depending on the method of microelement application, namely, in the aerial part, values ranging from 8.5 to 17.0 $mg \cdot kg^{-1}$ in the case of variants in which Zn was applied in the soil or on the seed, and high values ranging from 66.6 to 112.6 $mg \cdot kg^{-1}$ in the case of variants in which Zn was applied on the plant. The phenomenon was also similar in the case of roots, with close values between the control and the variants of application of Zn in the soil or on the seed. The fact that the highest content was registered in the roots of plants sprayed with Zn demonstrates that the trace element also circulated from the leaves to the roots.

Tabele 7. Microelements content ($mg \cdot kg^{-1}$) of maize plants (aerial part)

Collect date	Variant	Zn	Cu	Fe	Mn
44 days after seeding	Control	30.1 c	5.53 b	61.56 a	65.47 a
	0 Zn	12.5 a	3.80 a	62.53 a	57.53 a
	Zn in soil	21.6 b	3.17 a	60.57 a	38.76 a
	Zn on seed	12.4 a	3.10 a	62.93 a	50.43 a
	Zn on plant	112.6 d	4.00 a	61.87 a	53.43 a
58 days after seeding	Control	26.0 b	4.73 b	71.4 a	65.16 a
	0 Zn	8.56 a	3.06 a	70.5 a	38.47 a
	Zn in soil	17.0 ab	2.57 a	63.23 a	37.50 a
	Zn on seed	9.13 a	2.63 a	63.60 a	37.07 a
	Zn on plant	66.6 c	2.63 a	68.60 a	41.63 a

The phenomenon described in the absorption of Zn in plants also occurred in the case of Cu, but at a lower intensity. Both in the aerial part and in the roots, the highest Cu content was determined in the control plants. Differences in content between the control and the other variants are statistically ensured. Although there were no statistically significant differences between the contents of Fe and Mn in the aerial part of the plants, the phenomenon of the decrease of microelements in the variants trated with P and Zn is also preserved in this case on a smaller scale.

Table 8. Microelements content (mg·kg^{-1}) of maize plants (root part)

Collect date	Variant	Zn	Cu	Fe	Mn
58 days after seeding	Control	30.67 a	26.97 b	934 a	79.03 ab
	0 Zn	27.23 a	15.40 a	3193 b	113.7 c
	Zn in soil	28.60 a	17.10 ab	2250 b	75.2 ab
	Zn on seed	27.20 a	17.10 ab	2212 b	55.5 a
	Zn on plant	49.20 b	19.1 ab	961 a	106.5 bc

Instead, in the root there was a significant accumulation of Fe in the plants from the variants treated with P and Zn and at the same time a significant decrease in the Mn content.

CONCLUSIONS

For the biofortification of plants with Zn, the application of Zn on the seed was the most effective in terms of plant development (height), while the application of Zn in the soil was statistically the most effective in terms of green and dry mass, with values comparable to those of the application variant of the microelement on the seed.

The treatments applied to the soil material in the vegetation pots consisted of the administration of $CaCO_3$ and P. Zn was administered both in the soil, on the seed and on the plant in the different experimental variants. At the conclusion of the vegetation period, the treatments resulted in an increase in soil reaction and $CaCO_3$ content and a decrease in total and mobile Nt, N-NO_3, P_{AL}, K_{AL}, and microelement (Zn, Cu, Fe, Mn) contents due to their absorption by plants.

A different absorption of nutrients in the plant was recorded in the variants treated with $CaCO_3$, P and Zn applied to the soil, in the sense of a decrease in the absorption of N, Ca, Mg, Cu, Fe, Mn, partially and Zn and an increase in the absorption of P and K. The highest contents of macro- and micronutrients were recorded both in the aerial part and in the roots, in variant where Zn was applied directly on the plant.

After completing the study, it can be concluded that the administration of the microelement both in the soil and on the seed are the optimal methods for biofortifying maize plant with zinc, with nearly equal performance.

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