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DATA ON THE EFFECT OF ZINC ON THE GERMINATION AND GROWTH OF *Zea mays* L.

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Abstract. *Our study aimed to evaluate the effects of different concentrations of zinc on Zea mays L. cv. Turda 200 seedlings in the first phases of development. For this, in the spring we treated corn caryopsis, and placed it into germinators with different concentrations of Zn, in the form of zinc acetate, namely: 0.2%, 0.4%, and 0.8%, during the germination period. After 10 days after the beginning of the experiment, we concluded that the germination rate was not influenced by the presence of Zn in the soil, and Zn also acted as a trace element in the corn in the first ontogenetic phases.*

Keywords: *caryopsis, heavy metals, maize, monocots, seedling growth, zinc acetate.*

INTRODUCTION

Maize is part of the *Poaceae* family (Săvulescu 2007). It is an annual plant, originally from Central America, introduced to Romania in the 17th century (Pârvu 2006) and cultivated throughout the country, being an important food and fodder grain (Săvulescu 2007). It is considered one of the most important cereals in Romania and beyond (Ghețe et al. 2018). Zinc is a component of soil and is a necessary element for plant growth (Kaur & Garg 2021). When the conditions in the soil allow heavy metals to pass into the soil solution, their high concentrations in the soil present a direct risk of polluting the plants that absorb them (Gămăneci & Căpățână 2011). The distribution of heavy metals in the topsoil, up to a depth of 5 cm, is particularly important because of their bioavailability to plants and the risk of entering the food chain (Cordoș et al. 2007). Their presence in the deeper layer, up to 30 cm, reflects historical pollution, accidental infiltrations, and improper land management (Cordoș et al. 2007). The traditional use of zinc as a fertilizer (Cakmak 2007) and fungicide (Dordas 2008, Chavez-Dulanto et al. 2018, Cabot et al. 2019) for crop treatments, together with the combined effect of soil properties (pH, the amount of organic substances) and with increased humidity, favors the accumulation of zinc in agricultural soils. Excessive Zn amounts of up to 66400 mg·kg^{-1} (England), 80000 mg·kg^{-1} (USA), and 16000mg·kg^{-1} (Greece) were determined in the soils around the mining areas for the extraction of complex sulfides and the industrial areas for the processing of these ores (Kabata-Pendias 2010). In the Geochemical Atlas of Europe, the average Zn content of 52 mg·kg^{-1} was established for 845 samples collected from surface soils (Salminen et al. 2005). According to Malle (1992), in agricultural soils, zinc is largely unevenly distributed, and its content varies between 10 and 300 mg·kg^{-1}, with the lowest zinc values found in sandy soils and the highest in calcareous soils and organics, while Kabata-Pendias (2010) indicate an average zinc content for world soils of 64 mg·kg^{-1}. LUCAS data (Land Use/Cover Area Frame Survey) show that excess of zinc occurs in agricultural lands in more than 20% of NUTS regions (Nomenclature of Territorial Units for Statistics, created by the Statistical Office of the European Union - Eurostat), recording values exceeding the threshold concentration so that Zn pollution exists only in isolated cases in agricultural soils of the European Union (Toth et al. 2016).

The cases of heavy metal pollution investigated in Romania mainly refer to soils in industrial or urban areas, and agricultural soils have been less studied from this point of view. Thus, in the soils around the non-ferrous ore processing units, amounts of Zn accumulated up to 1378 mg·kg^{-1} (Baia Mare), 400 mg·kg^{-1} (Zlatna), 2010 mg·kg^{-1} (Copşa Mică) (Răuţă et al. 1995). According to the ICPA soil monitoring report (Dumitru et al. 2011), in 670 agricultural sites in Romania, the average zinc content was 87.34 mg·kg^{-1} soil, of which: for arable land, the average was 84 mg·kg^{-1} and for orchards, the average was 104 mg·kg^{-1}. Of the 670 analyzed sites, 79% had normal zinc contents, 20% were between the normal value and the alert threshold for sensitive use, values above the alert threshold (> 300 mg·kg^{-1}) were determined in 3 sites, and above the intervention threshold (> 600 mg/kg) in 2 sites. The toxicity of zinc varies greatly depending on many factors: plant species, plant age, metal concentration, exposure time, interaction with other ions in the environment, and nutrient composition of the environment (Hussain et al. 2022). Zinc toxicity is rare on lands without human activity (Băjescu & Chiriac 1984). For plant growth, Zn is essential at low concentrations and becomes toxic at higher levels (Wintz et al. 2002), producing necrosis and chlorosis (Manara 2012). Excess zinc in plants can have a negative influence on various elements of the photosynthetic apparatus - pigment biosynthesis, light capture, electron transport, CO_2 assimilation, and Calvin cycle enzyme activity (Sagardoy et al. 2010). Other manifestations of zinc toxicity encountered in crops are mentioned in the specialized literature: delay/inhibition of plant growth, reduction of chlorophyll synthesis, reduction of agricultural production (Rout & Das 2003, Yadav 2010), deficiencies of some elements, reduction of photosynthesis rate and transpiration (Vassilev et al. 2011), reduction of crop quality (Baran 2013, Li et al. 2013). Furthermore, Zn inhibits growth and alters the morphology and metabolism of soil microorganisms (Olaniran et al. 2013). As a rule, toxicity symptoms become visible at leaf zinc concentrations greater than 300 $mg \cdot kg^{-1}$, although some crops show toxicity symptoms at concentrations lower than 100 $mg \cdot kg^{-1}$ (Chaney 1993), and toxicity thresholds can have highly variable values even within the same species. Although excess Zn alters plant development (Baran 2012), some plants have evolved the ability to grow in environments with high concentrations of Zn in aerial parts, known as hyperaccumulators (Barbosa et al. 2017). According to Reeves et al. (2018), 28 Zn hyperaccumulating plant species have been described, most of them belonging to the *Brassicaceae* family. Thus, assisted phytoremediation technologies can be implemented (Zeremski et al. 2021). On the other hand, some studies reflect the potential of rhizobacteria (*Bacillus*, *Proteus*, *Pseudomonas* species) in the bioremediation of crops (maize, wheat) under zinc stress, their action reducing the negative effects of oxidative stress caused by zinc toxicity (Islam et al. 2014).

Based on the above-mentioned, we aimed to establish if the zinc from the substance zinc acetate has an inhibitory effect on the maize from the variety Turda 200, and to compare our results with the literature.

MATERIAL AND METHODS

In our experiments, early hybrid *Zea mays* L. cv. Turda 200 caryopses were used as plant material, which was placed in the spring period, for germination in four plastic, transparent casseroles with 32/21 cm and h = 12cm dimensions. A soil substrate with h=3 cm was placed in each pot and 50 grains were distributed evenly. The experiment had a control variant (V_0), which was watered throughout the duration only with distilled water (DW), and for the other three variants, we used different concentrations of the active substance according to the experimental protocol presented in Table 1.

Table 1. Experimental protocol (Zn – Zn in the form of zinc acetate; L – length; No. – number; T– temperature).

Experimental types	Type of measurements	The measuring range
V_0 – distilled water (*control*)	Germination rate	24h 48 h 72 h 5 days 7 days 10 days
V_1 - 0.2 % Zn		
V_2 - 0.4 % Zn	Growth rates: Embryonic root L. Adventitious roots No. Stem L. Leaflets L. Leaflets No. Seedling size Dry weight	10 days
V_3 - 0.8 % Zn		

The substance used in the experiment was zinc acetate hydrate - Zn $(CH_3COO)_2$*2 H_2O, used as such. Zinc was used in germination as zinc-ammonium acetate (ZAA) and applied to the soil with anhydrous ammonia as a carrier to increase maize (*Zea mays* L.) productivity (Liu et al. 2006). The authors found that ZAA reached the level of cytokine ratios in root and/or shoot tissues of maize seedlings, suggesting a secondary regulatory effect of ZAA in the development of maize productivity. ZnO nanoparticles, biogenically synthesized, were also used as a nano-priming agent to improve the germination and growth parameters of maize (*Zea mays*) seedlings (Itroutwar et al. 2020). When testing the nanoparticles, the authors used zinc acetate as an ionic control.

Previously, we calculated the volume of soil in the pots, then we calculated and weighed the amount of active substance required for the volume of soil, and for each experimental variant separately, being subsequently dissolved in distilled water (100 ml), resulting in the three concentrations of Zn used: 0.2%, 0.4%, and

0.8%, respectively. On the first day, 100 ml of solution was added to the soil, containing all the zinc concentrations related to the respective variant, and later, during the experiment, a total of 150 ml/lot of distilled water was added for each experimental variant. Cultures were maintained at 23° ± 2°C, naturally illuminated. When selecting the concentrations used for the experiment, we considered the normal value of the Zn concentration in the soil, which is 100 $mg \cdot kg^{-1}$, the value considered alert thresholds of 300 – 700 $mg \cdot kg^{-1}$, and the values for intervention thresholds of 600 – 1500 $mg \cdot kg^{-1}$ considered in risk situations, according to Order no. 756/1997, in Romania.

Applying 100 ml of the solution to the base, containing the concentration corresponding to each experimental variant on the first day of the experiment, constituted the abiotic stress to which the embryos were subjected.

After 10 days we measured the growth indices, then all the seedlings, depending on the experimental type, were placed in the desiccator, at 115 ºC, for 3 days, after which the dry substance was weighed.

RESULTS

The germination process of corn caryopses started 48 hours after they were placed in the soil substrate and continued for up to 7 days even though the experiment lasted 10 days (Fig. 1). After 5 days, the germination percentage was between 98% in the batch treated with distilled water (V_0) and in the batch treated with 0.2% Zn (V_1) and 100% in the batches treated with higher concentrations of zinc (V_2 and V_3), which were preserved until the end of the experiment (Fig. 1).

Regarding the growth and development indices of maize seedlings, increases in the lots treated with higher concentrations of zinc were noticed, respectively 0.4% and 0.8% (V_2 and V_3), compared to the values of the control lot. Thus, in the corn seedlings belonging to the V_1 lot (0.2% Zn), a slight inhibition was noted compared to the control in terms of the average value of the waist, and from a statistical point of view, the negative differences are distinctly significant, both for the height of the seedling, as well as for the number of leaflets, respectively significant for the length of the leaflets (Table 2).

The highest values for the length of the coleoptile, the length of the leaves, respectively the height of the seedlings were recorded in the lot treated with the concentration of 0.4% Zn (V_2), considered critical, with distinctly statistically significant positive differences for the length of the stem, respectively significant differences regarding the length of the leaves and the waist of the seedlings. These results indicate a strong stimulation of caulogenesis starting from 0.4% zinc concentration. In the case of some growth indices (Table 2), we can notice that the average values of differences compared to the control, are extremely small, from a mathematical perspective (0.21) (see V_1 - the number of leaflets), but they are distinctly significant compared to this, that is, with a higher degree of significance than the mean value of 1.91 cm, in the case of V_1, in terms of the length of the leaflets, where, even if mathematically, the mean values in centimeters are statistically higher, they are only significant.

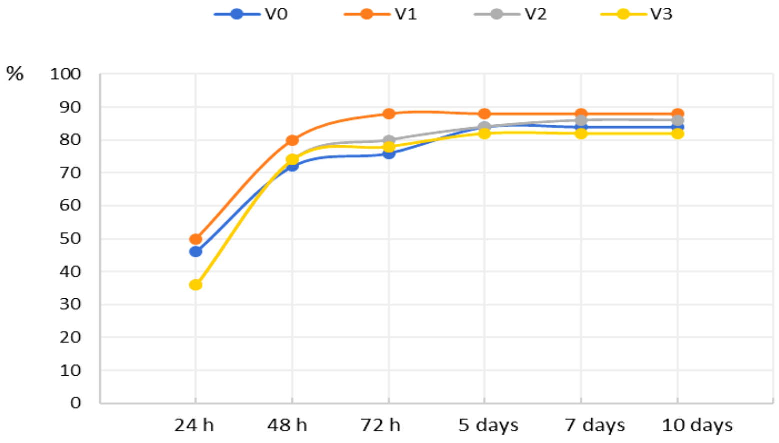

Figure 1. Monitoring the germination rate of maize caryopses (*Zea mays* L.), on the following experimental variants: V_0 – distilled water (*control*); V_1 – 0.2% Zn; V_2 – 0.4% Zn; V_3 – 0.8 % Zn, 10 days after setting up the experiment, at different time intervals (h-hours).

Regarding the dry weight (Table 3), the lightest seedlings among the groups treated with Zn were those from the group treated with the zinc concentration considered critical (V_2 - 0.4%), but the value is higher than the value of the control lot. The lot treated with the highest concentration of Zn (V_3 -0.8%) also recorded the highest biomass value, reaching 168%, a value that is reflected in the maximum number of plants. These data suggest an increase in maize yield in the presence of increased zinc concentrations.

Table 2. Statistical processing of the growth index values in maize (*Zea mays* L.), 10 days after putting the caryopses to germinate on different experimental variants: V_0 – distilled water; V_1 – 0.2% Zn; V_2 – 0.4% Zn; V_3 – 0.8% Zn.

Experi-mental types	Average ± standard deviation						Difference from the control / Difference signification (p)					
	Embryonic root L.(cm)	*Adventitious roots No.*	*Stem L. (cm)*	*Leaflets No.*	*Leaflet L. (cm)*	*Seedling size (cm)*	*Embryonic root L.(cm)*	*Adventitious roots no.*	*Stem L.(cm)*	*Leaflets No.*	*Leaflets No. (cm)*	*Seedling size(cm)*
V_0	17.65±5.68	3.96±1.02	9.30±2.30	2.31±0.51	9.06±4.37	39.79±7.04	-	-	-	-	-	-
V_1	18.25±6.06	3.90±1.23	8.92±2.96	2.10±0.59	7.45±3.59	37.01±8.82	0.60 0.76^{ns}	-0.06 0.79^{ns}	-0.38 0.70^{ns}	-0.20 0.07**	-1.61 0.31*	-2.78 0.09**
V_2	19.42±5.63	3.84±1.11	11.41±2.0	2.26±0.44	11.43±4.38	42.02±8.30	1.77 0.33*	-0.12 0.58^{ns}	2.11 0.01**	-0.05 0.63^{ns}	2.37 0.15*	2.23 0.15*
V_3	19.81±4.72	4.22±1.23	10.29±2.1	2.36±0.56	9.90±3.64	41.69±7.36	2.16 0.17*	0.26 0.25*	0.99 0.17*	0.05 0.62^{ns}	0.84 0.54^{ns}	1.90 0.19*

Note: ***$p<0.01$ - very significant; **$p<0.1$ - distinct significant; *$p<0.5$ - significant; ns $p>0.5$ - no significant.

Table 3. The dry weight of maize seedlings (*Zea mays* L.), 10 days after putting the caryopsis to germinate on the following experimental variants: V_0 –distilled water; V_1 – 0.2% Zn; V_2 – 0.4% Zn; V_3 – 0.8 % Zn.

Experimental types	Dry weight (g)
V_0	1.479
V_1	1.924
V_2	1.844
V_3	2.484

DISCUSSION

The high values of germination rate were recorded at all experimental variants, reflecting the fact that zinc caused stimulation of germination in the caryopsis of *Zea mays*. In addition, the stimulatory effect of zinc on corn plants is also reflected in the maximum percentage of germination in a short period (5 days). Nawaz et al. (2021) reported an increase in germination indices in maize by applying different treatments of caryopsis priming in Zn or Se. Similarly, Mahmood et al. (2005), studying the effects of zinc toxicity on germination and seedling growth in maize, clearly found that germination was not inhibited by different concentrations of Zn (3 ppm, 6 ppm, 9 ppm, 12 ppm), this being greater than 98% in all experimental variants, and Nciizah et al. (2020) found that at Zn concentrations of 0.01%, 0.05% and 0.1%, the percentage of maize germination was improved.

Significantly positive correlations were established in maize seedlings between the increased level of Zn and the length of the embryonic root (Table 2), which suggests stimulation of rhizogenesis in all experimental groups. However, the average of adventitious roots was lower in variants V_1 and V_2 compared to the control but statistically insignificant. In the case of the variant treated with 0.8% Zn (V_3), the root system was better developed, with statistically significant positive differences compared to the control group, both in terms of the length of the embryonic roots and the number of roots adventitious. As a result, zinc applied to hybrid maize plots in our experiment served as a trace mineral for this plant, regardless of concentration. However, necrosis was observed at the tip of the leaves in some maize seedlings of the lots where we applied higher concentrations of zinc (V_2 and V_3); similar effects were also reported by other authors such as Mehra & Farago (1994) and Ye et al. (1997).

Partially different results were reported by Baran (2012; 2013), who established the medium (470 mg Zn/kg) and high (1167 mg Zn/kg) critical toxicity levels for maize, levels that caused a decrease in yield by 20% and 50%, respectively, concluding that maize is less sensitive to zinc soil pollution than other cereals. Nciizah et al. (2020), treating maize corms with different concentrations of Zn (0.01%, 0.05%, 0.1%, 0.5%), reported an increase in root length, stem length, and implicitly of seedling height up to the concentration of 0.1% Zn, beyond this value the differences are statistically insignificant. Mahmood et al. (2005) observed inhibitory effects on stem length growth in *Zea mays* L. cv seedlings Neelum, with increasing zinc concentrations (6 ppm, 9 ppm, 12 ppm), root length not being much influenced by varying zinc concentrations, significant negative differences being recorded only at 12 ppm Zn concentration. Wang et al. (2011), investigated the toxic potential of zinc on wheat plants (*Triticum aestivum* L.) under hydroponic culture conditions, finding a gradual inhibition, with increasing Zn concentrations, both in terms of germination rate (reduced by 47.9% at 250 mM Zn), as well as rootlet length and plumule elongation (100% inhibition at 250 mM Zn). According to our data (Table 2 and Table 3), it is evident that the toxic effect of zinc is insignificant on this monocotyledonous species and, in addition to the beans (Ilieș-Lulușa & Petruș-Vancea 2021), in maize, the effect of stimulation of zinc was stronger, a fact reflected not only in the percentage of germination but especially in the level of the length of the stem, the number of leaves and the height of the seedlings.

In our case, there is a discrepancy between indices such as stem length, the number of leaflets, and leaflet length, which showed inhibitions compared to the control, in variant V_1 (Table 2), with only 0.2% Zn and the dry weight weighed in this lot of seedlings (Table 3). The explanation would be that, in this case, the seedlings were small in size, but still, with high mineral content. On the other hand, the results obtained in the experiment proved increases in growth indices in corn at the concentration of 400 mg/dm^3, even if Borkert et al. (1998), from the concentrations we started at the beginning of the experiment, established the concentration critical toxicity of Zn on the development of *Zea mays* plants as being greater than 300 mg/dm3. The same authors established the critical toxic concentration of zinc for the studied dicotyledonous species, i.e., for peanut - 36 mg/dm^3 and 70 mg/dm^3 for soybean, therefore they concluded that dicotyledonous legumes were more sensitive to zinc toxicity than maize. Also, Baran

(2013) showed significant inhibition of root growth in *Zea mays*, more than inhibition of germination, at higher concentrations of zinc (over 750 $mg.kg^{-1}$), also demonstrating that maize is less sensitive to zinc concentrations. The level of tolerance to high zinc concentrations was higher in corn (monocotyledonous) compared to the dicotyledonous species of beans (Ilieș-Lulușa & Petruș-Vancea 2021), the toxicity is evident only from the perspective of dry weight, in beans.

The hypothesis from which we started in the present study turned out to be partially true, so Zn also acted as a trace element in certain concentrations. The germination rate was not influenced by the presence of Zn in the soil, regardless of the concentrations we tested. Even though not all the growth indices in corn showed increases compared to the control, by increasing the concentration of Zn, the dry weight showed increases.

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