THE EFFECT OF ZINC ON GERMINATION AND SEEDLINGS GROWTH OF *ERUCA SATIVA* Mill.

EFECTUL ZINCULUI ASUPRA GERMINAȚIEI ŞI CREŞTERII PLANTULELOR LA *ERUCA SATIVA* Mill.

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Abstract. *The effect of zinc on seed germination and growth in the first ontogenetic stages in the species Eruca sativa was investigated. Zinc was used as sulphate solutions, in five different concentrations: 50 mg/L, 100 mg/L; 150 mg/L, 200 mg/L; 250 mg/L. We analyzed the following indicators: the percentage of germinated seeds; the length of root, the length of the hypocotyl, the tolerance index, the seedling vigor index, water content and dry matter content of the seedlings. The following effects were found: the insignificant modifications of the germination percentage; the significant delay of the growth in length of the root and of the hypocotyl; the decrease of the tolerance index, of the seedling vigor index and of the water content.*
Key words: zinc, germination, tolerance index

Rezumat. *S-a investigat efectul zincului asupra germinației semințelor şi creşterii în primele stadii ontogenetice la Eruca sativa Mill. Zincul s-a folosit sub formă de sulfat, în 5 concentrații diferite: 50 mg/L, 100 mg/L; 150 mg/L, 200 mg/L; 250 mg/L. S-au luat în studiu următori indicatori: procentajul de germinație, lungimea rădăcinii, lungimea hipocotilului, indicele de toleranță, indicele de vigoare a plantulelor conținutul de apă şi de substanță uscată din plantule. S-au constatat următoarele efecte: modificări nesemnificative statistic ale procentajului de germinație; reducerea semnificativă a lungimii rădăcinii şi a lungimii hipocotilului; scăderea indicelui de toleranță, a indicelui de vigoare a plantulelor şi a conținutului de apă.*
Cuvinte cheie: zinc, germinație, indice de toleranță

INTRODUCERE

Zinc is a microelement involved in the metabolism of some groups of substances (glucides, lipids, protein, auxins, etc.), in germination, growth, photosynthesis, etc. (Davidescu *et al*., 1988; Rout and Das, 2003; Tsonev and Lidon 2012), but in high concentrations it becomes toxic (Rout and Das, 2003). Symptoms of toxicity were visible in concentrations of zinc from leaf, higher than 300 mg/kg dry material or less than 100 mg/kg dry material (Chaney, 1993 and Marschner, 1995 quoted by Broadley *et al.*, 2007 and Malecka *et al*., 2012). As symptoms of zinc toxicity are quoted: delay/inhibition of plant growth, reduction of chlorophyll synthesis, reducing the rate of the photosynthesis, chlorosis, etc (Rout and Das,

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2003; Krusdar *et al.,* 2004; Broadley *et al*., 2007). The occurence of the toxicity phenomena was reported on land affected by human activities: depositing of the industrial and domestic waste; excessive application of fertilizers and pesticides that contain zinc, etc. (Broadley *et al*., 2007; Vasiliev *et al.,* 2011). Romanian legislation provides that the maximum permissible limit for the zinc content from soil is 100 mg/kg; the alert threshold is 300 mg/kg for the land with sensible use and 700 mg/kg for the land with less sensible use (Ordin nr. 756/1997 pentru aprobarea Reglementării privind evaluarea poluării mediului).

Eruca sativa Mill. (Brassicaceae family) with the updated name *Eruca vesicaria* (L) Cav. ssp. sativa (Mill.) Thell. is an annual herbaceous species, 20-60cm high, of Mediterranean origin (Săvulescu, 1958; Sârbu *et al.,* 2013). In Romania, it is spread sporadically in the field area and in the hills (Sârbu *et al*., 2013). The young leaves are used in alimentation. It is considered to be a good source of compounds with important nutritional value. A series of studies have underlined the chemical composition of the leaves (protein, glucides, mineral salts, vitamins, fatty acids, etc) (Bukhsh *et al.,* 2007; Nurzyńska-Wierdak, 2015) and seeds (Bukhsh *et al.,* 2007) and the potential medical properties (to prevent cancer, gastro-protective, antioxidant, etc.) (Michael, 2011; Saleh *et al.*, 2016). *Eruca sativa* has the ability to tolerate high metals (zinc, copper, cadmium, lead) in the environment of growth (Saleh, 2001; Zhi *et al*., 2015) and to accumulate some heavy metals (lead, cadmium) (Saleh, 2001).

This paper has as purpose the investigation of the effect of zinc on seed germination and growth of seedlings in the species *Eruca sativa* Mill.

MATERIAL AND METHODS

The biological material, was represented by seeds of *Eruca sativa* Mill. purchased from a shop specialized in seed marketing. The seeds were disinfected for 5 minutes with 1 % sodium hypochlorite solutions and afterward washed several times with distilled water. The seeds were placed to germinate in Petri dishes, on a filter paper humidified with distilled water (a control variant) and zinc sulphate solutions (treatment variants). The concentration of zinc from solutions were: 50mg/L (V1), 100mg/L (V2); 150 mg/L (V3), 200 mg/L (V4), 250 mg/L) (V5). Plates were kept at laboratory condition: at room temperature(20^{0}C - 24^{0}C), a photoperiod corresponding to the month of May, 2017. The total duration of the experiment was of 7 day after mounting. For each variant we used 4 replications, each replication with 15 seeds Germinated seeds were counted each 24 hours. After 7 days the root length and hypocotyl length was measured at a number of 40 seedlings for each experimental variants; the water and dry matter content of seedlings was determined by the gravimetric method (Boldor et. al., 1983). The following indicators were analyzed: the percentage of germinated seeds (at 1- 4 day, 7 days); the length of the root, the length of the hypocotyls and the seedlings; the tolerance index; the seedling vigor index, the water and dry matter content of seedlings. The tolerance index of heavy metals (TI) was calculated by the formula described by Iqbal and Rahmati, (1992) (Ahmad *et al*., 2012). The seedling vigor index was calculated by the formula described by Moradi *et al*., 2008. Also, the ratio of root length and hypocotyl length was calculated. The results presented in figure and tables are expressed as mean value ± standard error (for the germination percentage n = 4; for the morphological indicators n = 40). The data obtained from the germination percentage, and the morphological indicators were statistically interpreted. The unifactorial Anova test followed by the Tukey test (α = 0.05) was used in order to test the differences between means (Zamfirescu and Zamfirescu, 2008).

RESULTS AND DISCUSSIONS

During the first 4 days after assembling the experiment, the *percentage of germination* presented average values smaller in the variants of treatment than in the control. The lowest average values were registered in the variant of treatment V5; in this case, the difference from the control was reduced gradually from the first day until the fourth day. After seven days, the *percentage of germination* registered a slight increase in value comparing with the control in the variants V1-V4 and with a decrease in value, respectively, by 10.35% in the variant V5; these modifications are insignificant statistically (p>0.05) (Figure 1). The results obtained suggest the fact that the germination of the seeds of *Eruca sativa* is tolerant to zinc in the concentrations used, fact signalled by other authors as well, but for other concentrations of zinc (Ozdener and Kutbay, 2009; Zhi *et al.*, 2015).

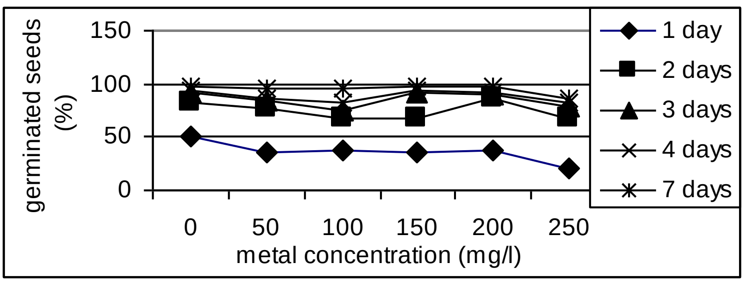

Fig. 1 The dynamics of germination at *Eruca sativa*

The length of the root (LR), *the length of the hypocotyl* (LH) and *the length of the seedling* (LS) registered a decrease in value comparing with the control in all the variants of treatment (by 15.58 % - 86.91 % comparing with the control in the case of the root; by 11.42 % - 62.35 % in the case of the hypocotyl; by 14.05 % - 76.93 % in the case of the seedling). From the statistic point of view, the reduction was significant ($p \leq 0.05$) in the variants V2-V5 in the case of the root and in all the variants in the case of the hypocotyl and seedling (tab. 1). The unfavourable effect was very acute in concentrations of 200mg/L and 250mg/L zinc in the environment of growth. The proportion between the length of the root and the length of the hypocotyl (LR/LH) reduced in value gradually with the increase of the concentration of the metal, the reduction being significant in the variants V3-V5 (tab. 1). The morphological parameters of the seedlings exposed to the treatment with zinc varied inversely proportional with the concentration of the metal.

Table 1

The analyzed indicators

The indicator	The variants					
	Control	V1 (50mg/l)	V2 (100mg/l)	V3 (150mg/l)	V4 (200mg/l)	V5 (250mg/l)
LR (mm)	34.9±1.61	29.37±1.4	26.25±1.58*	17.02±1.75*	8.6±1.09*	4.57±0.72*
LH (mm)	23.82±0.73	21.1±0.62*	18.05±0.81*	15.32±0.63*	13.32±0.65*	8.97±0.47*
LS(mm)	58.72±1.66	50.47±1.6*	44.3±1.8*	32.22±2.09*	21.95±1.58*	13.55±1.12*
LR/LH	1.53	1.43	1.57	1.06*	0.60*	0.47*
IT (%)	-	84.15	75.21	48.76	24.64	13.09
ISV	567.39	478.8	420.8	317.6	215.34	116.99
W (g%)	90.5	89.76	87.18	87.4	86.24	82
DS (g%)	9.5	10.24	12.82	12.60	13.76	19.80

Note: * indicates significant differences (Tukey test, $p<0.05$)

The reduction of the process of growth in length for the root and the hypocotyls in the case of the treatment with zinc was also reported by other authors: Ozdener and Kudbay (2009), Al-Quariny (2010) at *Eruca sativa*; Çavuşoğlu *et al.*, (2009) at *Phaseolus vulgaris;* Sharma *et al.,* (2010) at *Cicer arietinum;* Ashagre *et al.,* (2013) at *Lycopersicon esculentum*. The results obtained have underlined the fact that the process of growth for the seedlings (especially the growth in length of the root) comparing with the process of germination was more sensitive to the treatment with zinc in the concentrations used. Similar observations have been reported also by other authors: Ozdener and Kudbay (2009), at *Eruca sativa;* Li *et al.,* (2005), at *Arabidopsis thaliana* for zinc and other heavy metals. The effect noticed on the process of incipient growth of the seedlings of *Eruca sativa* would be probably due to the metabolic and physiological disorders caused by the ions of zinc in excess in the solutions used for treatment. The ions of zinc in excess induce the appearance of the oxidative stress (Jain *et al.,* 2010), the chromosome aberrations (Liu *et al.,* 1996; Jain *et al.*, 2010); they inhibit the cell division and elongation (Khudsar *et al.,* 2004), the growth of the root (Liu *et al.,* 1996), the growth of the plants; they determine the browning of the root (Vassilev *et al.*, 2011).

Index of tolerance (IT) presented a gradual decrease in value, with the increase of the metal concentration. Values of IT less than 50 % were registered in concentrations between 150mg/L – 250mg/L (tab. 1). *Index of seedling vigour* (ISV) registered a decrease in value gradually with the increase of the zinc concentration in the solutions used for the treatment (tab. 1). For concentrations between 150mg/L - 250mg/L, the rate of reduction comparing with the control for the ISP was between 44.05 % - 79.33%. The decrease of the index of tolerance and the index of vigour with the increase in the zinc concentration was reported by Ashagre *et al*. (2013) in the species *Lycopersicon esculentum*.

The water content (W) in the seedlings presented a decrease in value in the variants of treatment comparing with the control (tab. 1). Similar effects were reported also by Singh *et al.* (2007) in the seedlings of *Triticum aestivum* in the case of the treatment with concentrations of 5-100 mg/L copper. Water is essential for germination and growth; the results obtained suggest a possible disorder of the processes of absorption and use of water in the germination stage and post germination. *The content of dry substance* (DS) presented reversed amplitudes comparing to those described in relation with the water content (tab. 1).

CONCLUSIONS

1. *Eruca sativa* has the ability to germinate and grow in the first ontogenetic stages, in the presence of some moderate and high concentrations of zinc; the level of tolerance to zinc was higher in the phase of germination.

2. The process of growth during the first ontogenetic stages was affected proportionally with the increase in the metal concentration.

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