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THE EFFECT OF ZINC ON GERMINATION IN SPECIES OF THE GENUS *ECHIUM* (FAMILY BORAGINACEAE)

Alexandra-Roxana SAVA[1], Maria-Magdalena ZAMFIRACHE[1], Anișoara STRATU[1*]

[1]Departament of Biology, Faculty of Biology, "Alexandru Ioan Cuza" University of Iasi, Romania

*Correspondence:
Anișoara STRATU
anisoara.stratu@uaic.ro

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Abstract: The effect of zinc on seed germination in two species of the *Echium* genus (*Echium russicum* J.F. Gmelin and *Echium vulgare* L.) was investigated. Zinc was used as sulphate in three different concentrations: 100 mg/l; 200 mg/l și 300 mg/l; the treatment was applied to the seeds before germination, the exposure time being short (4 hours and 8 hours). Seed germination was monitored daily for a period of 14 days. The effect of treatment on seed germination in the test species was assessed by calculating four germination indicators: final germination percentage (FGP), germination index (GI), mean germination time (MGT) and coefficient of rate germination (CRG). The results show the following: the seeds of *Echium russicum* germinate faster than those of *Echium vulgare*; statistically insignificant changes in the indicators associated with germination, except for GI in *Echium vulgare* for the 8 hours exposure time; reduction of germination percentage (in both species) and increase in germination time (more evident in *Echium vulgare*) with increasing exposure time. The test species showed tolerance throughout the germination process to the zinc concentrations used.

Keywords: zinc, *Echium russicum*, *Echium vulgare*, germination indices

1. Introduction

Zinc belongs to group II-B of the periodic table of elements and is a transition metal.

Zinc is a microelement involved in many biochemical (enzyme activity, metabolism of some groups of organic compounds) and physiological processes (growth and development, photosynthesis, defense against diseases and pests, etc.) specific for plant life. The role of zinc for plants is described in literature by various authors (Rout and Das, 2003; Zamfirache, 2005; Sadeghzadeh, 2013; Wyszkowska et al., 2013).

According to Jain et al. (2010), zinc is the second most abundant transition metal in organisms after iron.

Although zinc is a micronutrient for plants, at high concentrations it can negatively influence biochemical and physiological processes (enzyme activity, cell division, germination, photosynthesis, plant growth and development) (Rout and Das, 2003; Jain et al., 2010). Toxicity limits vary according to species, ontogenetic phase, exposure time. According to Macniol and Beckett (1985), the upper toxicity level for zinc is between 100 ppm and 500 ppm.

Anthropogenic sources of environmental pollution with zinc are represented by industrial activities (mining/processing of ores), industrial waste disposal, road transport, agricultural activities (excessive application of pesticides and fertilizers), etc. (Vasiliev et al., 2011; Wyszkowska et al., 2013).

In Romania, according to the legislation in force (Ordin nr. 756/1997) the maximum allowed limit for the total zinc content in the soil is 100 mg/ kg. The alert threshold is 300 mg/ kg for sensitive land and 700 mg/kg for less sensitive land. For the intervention threshold, the following values are established: 600 $mg \cdot kg^{-1}$ for sensitive lands and 1500 mg/kg for less sensitive lands.

Some species of the genus *Echium* such as *Echium russicum* and *Echium vulgare* have been studied for their physiological response to stress conditions caused by the presence of heavy metals or their phytoremediation potential (Izmaiłow and Biskup, 2003; Dresler et al., 2014; Dresler et al., 2017a; Dresler et al., 2017b.; Jakovljević et al., 2019; Murtić et al., 2021).

Echium russicum J.F. Gmelin, syn. *Echium rubrum* Jacq., non Forssk. (Gibbs, 1972), syn. *Pontechium maculatum* (L.) Böhle & Hilger (Hilger and Böhle, 2000), syn. *Echium maculatum* L. (Sârbu et al., 2013) is a biannual, heliophytic, subthermophilous, xerophilous, neutrophilous, oligo-mesotrophic, therophyte-hemiterophyte species. (Sârbu et al., 2013). The species has melliferous, decorative and medicinal importance (Grințescu, 1960; Chwil and Weryszko-Chmielewska, 2007***;*** Moazzami Farida et al., 2020). *Echium russicum* is a species of community interest, being considered a good indicator of the state of conservation of dry and semi-dry steppe and forest grasslands (Mănoiu and Brînzan, 2013). It is included in Annexes II and IV of the Habitats Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora species, as well as in Annex I of Resolution 6 (1998) of the Berne Convention, revised in 2011 (https://eunis.eea.europa.eu/species/162097). According to Jakovljević et al. (2019) *Echium russicum* grows on both metalliferous and non-metalliferous soils and is considered a facultative metallophytic species. The results of the research carried out by the authors noted above with plants taken from sites with ultramafic soils in Serbia indicated the efficient absorption of zinc and copper from the soil and their translocation in the aboveground organs, a translocation which is considered a detoxification mechanism.

Echium vulgare L. is a hemiterophytic, biannual (or sometimes perennial), heliophilous, eurythermal, xero-mesophilic, oligo-mesotrophic, eurythermal species (Sârbu et al., 2013). It is a common species in Romania and has medicinal, melliferous, tanning importance (Grințescu, 1960; Eruygur et al., 2012). According to Dresler et al. (2014), *Echium vulgare* as a pseudometalophyte can grow on both uncontaminated and heavy metal polluted land; plant populations on heavy metal polluted land showed high tolerance to cadmium. Studies conducted in Poland on *E. vulgare* populations established spontaneously on lands with heavy metal waste from mining activity showed that plants growing on metalliferous lands produced smaller seeds with lower germination abilities (Dresler et al., 2017a). The root and stem of *Echium vulgare* accumulate heavy metals (Cu, Zn, Pb, Cd, Fe) and show a higher concentration of secondary metabolites (allantoin, shikonin derivatives), as a response to stress conditions caused by heavy metals in the growth environment. (Dresler et al., 2017b). Studies conducted in Bosnia and Herzegovina by Murtić et al. (2021) showed that *Echium vulgare* plants grown spontaneously on the land located in the vicinity of a steel mill accumulated heavy metals (Cr, Cd, Zn, Pb, Ni) in the above-ground parts, but the ability to remove/stabilize heavy metals in the polluted soil was decreased.

The present work aims to investigate the influence of zinc treatment on the germination process in *Echium russicum* J.F. Gmelin and *Echium vulgare* L. species.

2. Materials and methods

Plant material

The plant material was represented by seeds belonging to two species of the *Echium* genus: *Echium vulgare* (EA020) and *Echium russicum* (EA017) purchased from Jelitto Staudensamen GmbH in Schwarmstedt, Germany (https://www.jelitto.com/).

Experimental procedures

Zinc was used in three different concentrations (100 mg/l, 200 mg/l and 300 mg/l), in the form of aqueous zinc sulphate solutions [$ZnSO_4.7\ H_2O$].

The seeds were immersed in zinc sulphate solutions (treatment variants) and respectively in distilled water (control), for a period of 4 hours and 8 hours, respectively.

The treated seeds were transferred for germination in sterile Petri dishes, on filter paper moistened with distilled water (4 ml each). For each experimental variant, four replications were used, each replication with 10 seeds. The Petri dishes were kept under laboratory conditions (with the naturally day/night photoperiod characteristic of March-April, year 2021 and an average temperature of approximately 21°C) for a period of 14 days. During this experimental period the germinated seeds were counted at intervals of 24 hours.

Studied parameters

The effect of the treatment on the germination of the test species was evaluated by calculating 4 germination indicators, according to the formulas described by Islam and Kato-Noguchi, (2014): final germination percentage (FGP), germination index (GI), mean germination time (MGT) and coefficient of rate germination (CRG).

Statistical analysis

All results noted in the tables are expressed as mean value ± standard error. The data obtained for each indicator studied were statistically analyzed using the one-way ANOVA test and the Tukey HSD test (to test the difference between mean values). Differences were considered significant when the *p*-value was ≤ 0.05.

3. Results and discussions

The final percentage of germination (FGP), for the treatment with exposure duration of 4 hours, registered a slight value increase for all the concentrations used, in the case of the species *E. vulgare* (by 9% - 13.63%) and for the concentrations of 200mg /l (by 15.78%) and 300mg/l (by 21.05%) in the species *E. russicum*. Also, FGP recorded a decrease of 25% in the case of the concentration of 100mg/l in the species *E.russicum* (**Table 1, Table 2**).

For the treatment with an exposure duration of 8 hours, in both test species, FGP recorded lower values than in the control in all treatment options: by 17.25% - 31.04% in *E. russicum* and by 14.29% - 32.15% in *E. vulgare* (**Table 3, Table 4**).

From a statistical point of view, the recorded changes are not significant, a situation valid for each exposure period and for each test species (**Table 1-4**).

Regarding FGP, the results obtained in the present study for the control in the case of the *E. russium* species are in agreement with those presented by Nowak et al. (2020).

The mean germination time (MGT) presented (with few exceptions) slightly higher values in the treatment variants compared to the control (by 0.43% to 23.52%) for each test species and each exposure period. MGT recorded statistically insignificant changes ($p \geq 0.05$) (**Tables 1-4**).

Depending on the experimental variant, the seeds of the two test species germinated as follows: in *E. russicum* - after a period of time between 5.9 and 7.72 days, and in *E. vulgare* after 8.53 – 10.03 days. This fact denotes a faster germination of the seeds of *E. russicum*, compared to those of *E. vulgare*. The obtained results confirm the data reported by Nowak et al. (2020) regarding the fact that *E. russicum* germinates quite quickly.

For the treatment with the exposure duration of 4 hours, the germination index (GI) registered statistically insignificant changes, compared to the control ($p \geq 0.05$). In both test species, a slight value increase was found (by 9% - 16% in *E. russicum* and respectively by 9% - 37% in *E. vulgare*), compared to the control, with only one exception (the concentration of 100 mg/l in *E. russicum)* (**Table 1, Table 2**).

In the case of the treatment with an exposure duration of 8 hours, in both test species, GI recorded slightly lower values in the treatment variants, compared to the control: by 21.06% - 41.06% in *E. vulgare* and 21% - 37.5% in *E. russicum* (**Table 3, Table 4**). The changes were statistically significant ($p \leq 0.05$) at the concentration of 100 mg/l in the case of *E.vulgare* species (statistical $F = 4.5345$; $p = 0.024$). By increasing the exposure period, the GI decreases in both species.

The coefficient of the germination rate (CRG) showed a slight value reduction in most treatment variants compared to the control, which was statistically insignificant, a situation valid for both treatment periods and both test species (**Table 1-4**).

The results obtained practically indicate that the seeds of the two species of the genus *Echium* studied have the ability to germinate quite well after a short period of exposure (4 hours and 8 hours, respectively) to different concentrations of zinc (100mg/l, 200mg/ l, 300mg/l).

For the two exposure periods, by comparatively analyzing the results obtained from the treatment variants with the control, the following aspects were found: a tendency towards a slight stimulation of germination in both species, although the germination time was longer than in the control, for the exposure period of 4 hours; a tendency for a slight delay in germination and a decrease in the germination index in both species for the 8-h exposure period, accompanied by an increase in germination time, more evident in *E. vulgare.* In the case of the *E. vulgare* species for the concentration of 100mg/l, statistically significant differences ($p \leq 0.05$) were found for some indicators analyzed at the 4-hour exposure time, compared to the 8-hour exposure time: FGP (F statistic = 7.7143; $p = 0.0321$) and GI (F statistic = 6.0586; $p = 0.049$). These results indicate that increasing the exposure time to certain concentrations can affect germination in the test species.

Studies have shown that the seminal integument and the other tissues around the embryo constitute the main barrier against heavy metals, and in the stages following imbibition the embryo becomes sensitive to heavy metals (Li, 2005).

In the species *E. russicum* and *E. vulgare* the term seed is used in a broad sense; in this case it is actually the fruit called nucula. Nucula is trigonous, pronounced coarse with a flat base, 2-3 mm long in *E. russicum* and obliquely ovoid trigonous, coarse, with flat base 3-4 mm long in *E. vulgare* (Grințescu, 1960; Gibbs, 1972).

Germination tests performed by Izmaiłow and Biskup (2003) with *E. vulgare* seeds collected from two categories of plants (plants grown on heavy metal-polluted sites and control plants), using polluted soils as substrate showed good seed germination from the first category and the reduction of germination in the case of seeds from control plants. The authors suggest that the seeds of control plants are more sensitive to pollutants than the seeds from specimens grown on soils polluted with heavy metals (zinc, lead, cadmium). According to the authors, the fruit coat and seed coat are permeable to heavy metal ions.

The results obtained in the present study are in agreement with those reported by other authors regarding the effect of different concentrations of zinc on germination. In the specialized literature, the data reported on the influence of zinc (in the form of zinc sulphate) on germination are very different, depending on the species, variety, on the concentrations used, on the duration of the experiment (Ashagre et al., 2013; Menon et al., 2016; Stratu and Costică, 2018; Bezini et al., 2019).

Table 1: The effect of different concentrations of zinc on the indicators of the germination process in *Echium russicum* seeds - exposure time 4 hours.

Zn treatment (mg/l)	**FGP (%)**	**GI (seeds day^{-1})**	**MGT (day)**	**CRG**
0	47.5±10.35 (100)	0.85±0.2 (100)	6.25±0.68 (100)	16.77±2.34 (100)
100	37.5±7.5 (78. 94)	0.58±0.12 (62,23)	7.72±0.31 (123.52)	13.01±0.52 (77.57)
200	55±11.9 (115,78)	0.93±0.22 (109.41)	6.52±0.83 (104.32)	15.95±1.64 (95.11)
300	57.5±11.08 (121,05)	0.99±0.26 (116,47)	7.07±0.66 (113.12)	14.51±1.36 (86.52)

Results are means ± SE of the four replicates
Parentheses contain the percentage values of each parameter relatively to the control.

Table 2: The effect of different concentrations of zinc on the indicators of the germination process in *Echium vulgare* seeds - exposure time 4 hours.

Zn treatment (mg/l)	**PG (%)**	**GI (seeds day^{-1})**	**MGT (day)**	**CRG**
0	55±14.43 (100)	0.62±0.13 (100)	9.21±0.74 (100)	10.88±1.13 (100)
100	62.5±2.5 (113.63)	0.85±0.07 (137.09)	8.63±0.52 (93.70)	11.7±0.7 (107.53)
200	60±7.07 (109.09)	0.68±0.06 (109. 67)	10±0.26 (108.57)	10.01±0.26 (92)
300	62.5±7.5 (113.63)	0.72±0.1 (116.12)	9.25±0.48 (100.43)	10.88±0.51 (100)

Results are means ± SE of the four replicates
Parentheses contain the percentage values of each parameter relatively to the control.

Table 3: The effect of different concentrations of zinc on the indicators of the germination process in *Echium russicum* seeds - exposure time 8 hours.

Zn treatment (mg/l)	PG (%)	GI (seeds day^{-1})	MGT (day)	CRG
0	72.5±6.29 (100)	1.28±0.13 (100)	6.43±0.38 (100)	15.68±0.88 (100)
100	50±10.8 (68.96)	0.8±0.23 (62.5)	7.37±0.55 (114.61)	13.8±1.09 (88.01)
200	55±5 (75.86)	1.01±0.13 (78.90)	5.9±0.41 (91.75)	17.18±1.22 (109.56)
300	60±12.45 (82.75)	1.01±0.21 (78.90)	6.7±0.29 (104.19)	14.97±1.05 (95.47)

Results are means ± SE of the four replicates

Parentheses contain the percentage values of each parameter relatively to the control.

Table 4: The effect of different concentrations of zinc on the indicators of the germination process in *Echium vulgare* seeds - exposure time 8 hours.

Zn treatment (mg/l)	PG (%)	GI (seminte day^{-1})	MGT (day)	CRG
0	70±8.16 (100)	0.95 ±0.09 (100)	8.53±0.41 (100)	11.8±1.22 (100)
100	47.5±4.78 (67.85)	0.56*±0.05 (58.94)	9.3±0.11 (109.02)	10.74±0.25 (91.01)
200	60±5.77 (85.71)	0.75±0.06 (78.94)	9.34±0.65 (109.49)	10.87±1.55 (92.11)
300	55±2.88 (78.57)	0.62±0.07 (65.26)	10.03±0.63 (117.58)	10.09±1.30 (85.5)

Conclusion

Zinc in the concentrations used in the present study does not, in most cases, produce significant changes in the germination indicators analyzed in *E. russicum* and *E. vulgare* species, which suggests that the test species are tolerant to these concentrations.

The obtained results indicate that zinc, although a micronutrient, can adversely influence germination at certain concentrations and exposure times.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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