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THE INFLUENCE OF ZINC ON SEED GERMINATION AND SEEDLINGS GROWTH OF *SALVIA COCCINEA* BUCHOZ EX ETL.

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Abstract: The paper presents the results of a study regarding the influence of treatment with zinc (as sulphate solutions) in different concentrations (50 mg/l, 100 mg/l, 200 mg/l, 300mg/l, 400 mg/l, 500 mg/l, 600 mg/l and 800mg/l) on the seed germination and the growth in early ontogenetic stages of *Salvia coccinea.* We analyzed the following indicators: the percentage of germinated seeds at different intervals (24 - 168 hours); the length of root and hypocotyl (at 168 hours). The results underline the specific variations of the analysed indicators, depending on the concentrations used for the treatments of seeds. The treatment with zinc sulphate solution with concentration of 100 mg/l presents a tendency of stimulate the germination (with 10 % compared to control). The effect on growth varies depending on the concentration. A delay effect of growth in length of the root and hypocotyls and an inhibitory effect was found (very high concentrations inhibit the growth).
Keywords: *Salvia coccinea*, germination, growth of seedlings, zinc.

Introduction

Zinc is a microelement with multiple roles in the life of plants (Caramete et al., 1974; Davidescu et al., 1988; Rout and Das, 2003; Broadley et al., 2007; Tsonev and Lidon, 2012); in high concentrations it can be toxic (Malecka et al., 2012). It is the only metal represented in the six classes of enzymes (oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases) (Jackson et al., 1990; Broadley et al., 2007). Among the roles of the zinc presented in the specific literature, we mention: it interferes in the synthesis of the proteins and the acid nucleic, the metabolism of the carbohydrates (Caramete et al., 1974; Davidescu et al., 1988; Jackson et al., 1990; Marschener, 2012 and Hänsch and Mendel, 2009 quoted by Tsonev and Lidon, 2012); in tryptophan synthesis – a precursor of auxin (Burzo et al., 1999; Davidescu et al., 1988); in reducing nitrates (Caramete et al., 1974; Davidescu et al., 1988); it plays a role in the maintenance of the integrity and stability of cellular structures (Davidescu et al., 1988; Tsonev and Lidon, 2012); in the protection of the cells against the damages caused by the species reactive to oxygen (Cakmak, 2000 quoted by Malecka et al. 2012).

According to some information in the field, the normal content of zinc in plants varies between 10 – 150 ppm (Mulligan et al., 2001) or between 25 ppm - 150 ppm (Malik et al., 2011); in concentrations of 400 ppm it becomes toxic (Mulligan et al., 2001). According to Chaney and Marschner quoted by Broadley et al. (2007) and Malecka et al. (2012), symptoms of toxicity were usually visible when the concentration of zinc in the leaf was higher than 300 mg/kg dry material, even though some cultivated plants present symptoms of toxicity in concentrations in the leaf lower than 100mg/kg dry material.

The toxicity of zinc is higher on acid soils, with reduced content of calcium, organic matter and available phosphates (Woolhouse, 1983); on contaminated soils of waste

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coming from the human activity (mines, industry, agriculture – overuse of fertilizers and pesticides that contain zinc; spill of waste water, burning fossil fuels, etc.) (Broadley et al., 2007; Vassilev et al., 2011). Toxicity varies a lot according to a series of factors, among which: species, age, concentration of metal, time of exposure, interaction with other ions in the environment, the composition of the nutrients in the growing environment etc. As manifestations of zinc toxicity, there are mentioned: the phenomenon of chlorosis (Woolhouse, 1983; Rout and Das, 2003; Broadley et al., 2007); inhibition of plant growth, reducing the agricultural production, reduction of chlorophyll synthesis (Rout and Das, 2003; Broadley et al., 2007; Tsonev and Lidon, 2012); it affects the gas changes at the level of leaf (Vassilev et al., 2011). The effects caused by the zinc toxicity are less severe comparing to the ones caused by other heavy metals such as nickel, cobalt, cadmium, etc. (Woolhouse, 1983).

In tomatoes, the zinc in concentration of 50 mg and 100 mg/kg soil has a positive effect on the growth of plants; in concentrations of 150 mg, and 250 mg/kg soil, it influences negatively the growth of plants studied by means of the following parameters: length of root, shoot, total surface of the leaf, dry mass of the root, shoot (Vijayarengan and Mahalakshmi, 2013). In *Phaseolus vulgaris*, the treatment with zinc in concentration of 30 ppm and 70 ppm (for 7 days) reduces the percentage of germination by 30 % and 44 % respectively (Çavuşoğlu et al., 2009). In *Cicer arietinum* the treatment with solutions of zinc sulphate in concentration of 75 mM and 100 mM reduces significantly the germination of the seeds, after 72 hours; in case of seedlings, the content of chlorophyll pigments decreases, and the activity of some antioxidant enzymes is intensified after 15 days. (Sharma et al., 2010).

This paper wants to investigate the effect of the zinc treatment of different concentrations on the germination and growth in the first ontogenetic stages in the species *Salvia coccinea* (Lamiaceae family). *Salvia coccinea* is an annual species, of medium size (60 - 80 cm height), native in North America (Sonea et al., 1979). It is a decorative species for its flowers, being cultivated in the urban green spaces and in the private gardens. It presents flowering in stages, of long duration (from the beginning of summer until late autumn) (Sonea et al., 1979; Preda, 1989).

Materials and methods

As a biological material, we used seeds of *Salvia coccinea* Buchoz ex Etl. obtained from seed retailers (S. C. Unisem S. A). Nine experimental variants have been created: a control variant (with distilled water - the variant marked C) and eight variants of zinc treatments.

Zinc was used as sulphate solutions in a concentration of 50 mg/l (the variant marked V1), 100 mg/l (the variant marked V2), 200 mg/l (the variant marked V3), 300 mg/l (the variant marked V4), 400 mg/l (the variant marked V5), 500 mg/l (the variant marked V6), 600 mg/l (the variant marked V7) and 800 mg/l (the variant marked V8). In selecting the concentrations used for the experiment we started from the critical concentration of zinc in soil (400 mg/l) (according to Alloway, 1990; Beckett and Davis, 1979, quoted by www.cprm.gov.br /). The seeds were placed to germinate in Petri dishes, on a filter paper humidified with distilled water (a control variant) and zinc sulphate solutions (a treatment variants).

The plates were kept in a growth chamber (Snijders Scientific type), at 22°C (12h) – 24°C (12h), 60% relative humidity and a 12:12 hours photoperiod. The initial volume of distilled water or zinc sulphate solutions (at placing the seeds) was of 4 ml. During the experiment, germination substrate was wetted with distilled water (control variant) and sulphate of zinc solutions (treatment variants). For each variant, three replications (each with 30 seeds) were used.

The following indicators have been analyzed: the percentage of germinated seeds at different intervals (24 - 168 hours); the length of the root and hypocotyl (at 168 hours after the beginning of the experiment). The measurements (the length of the root and hypocotyl) were performed on 30 seedlings for each experimental variant. All the results presented in figures were expressed as mean value ± standard error. The data obtained from the length of the root and of hypocotyl were interpreted statistically. The unifactorial Anova test was used and in order to test the difference between averages the Tukey test was used (Zamfirescu and Zamfirescu, 2008; Microsoft Excel program).

Results and discussions

The germination of seeds. In *Salvia coccinea* germination is epigeous. The seeds germinate after 72 hours from the beginning of the experiment. After this period of time, the percentage of germination in the control registered an average value of 31.11 %, and in variants of treatment, average values between 1.11 % – 39.99 %. Higher values comparing with the control have been recorded in the variants V2 and V3 (Figs. 1; 2).

During the analysed period, the percentage of the germinated seeds grows progressively. At the end of the experiment (at 168 hours), the percentage of germination registered average values between 75.55 % - 82.22% in the treatment variants; in the control, it was registered an average value of 74.44% (Figs. 1; 2). A tendency to stimulate the germination with values between 1.49 % - 10.42 % was recorded; the effect of stimulation is more obvious in the case of concentration of 100mg/l, during the whole period studied.

The results obtained indicate the fact that the zinc in the concentrations used does not affect the germination; the seeds of salvia are able to germinate in the presence of some moderate and high concentrations of zinc in substrate.

Effects of stimulation of the germination in the case of treatment with zinc in different concentrations were also reported in other cultivated species: *Cicer arietinum* (after 72 hours) in concentrations of 10 mM and 25 mM (Sharma et al., 2010); *Cicer arietinum, Macrotyloma uniflorum, Vigna radiata, Vigna unguiculata* in case of concentrations of 10, 20, 50 ppm (Shivakumar and Thippeswamy 2012).

We consider the fact that the results obtained are due to the particularities of the seeds in the test species taken for study. In *Salvia coccinea* we noticed the fact that the seeds form after the imbibition, mucilage of opaque consistency. This phenomenon is called myxospermy (Western et al., 2000; Ifrim 2012), in the case of fruits the correct term is myxocarpy (Ifrim, 2012); the phenomenon is due to the presence of some structures that produce mucilages. This phenomenon is present also in other species of the genus Salvia (*Salvia aehtiopis, S. austriaca, S. nemorosa, S. multicaulis* etc.), in other species in the family Lamiaceae (Habibvash et al., 2007; Ifrim, 2012), as well as other species in other botanical families (Brassicaceae, Solanaceae, Linaceae, Plantaginaceae) (Western et al., 2000).

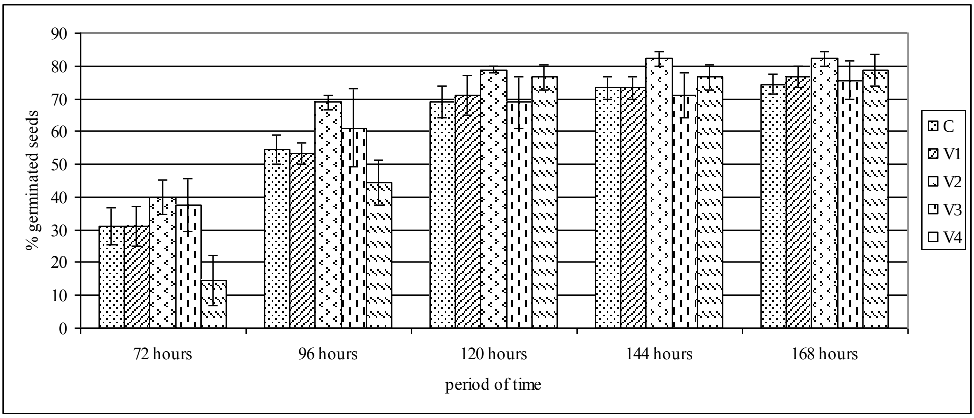

Figure 1. The percentage of the germinated seeds - control and the treatment variants (V1-V4)

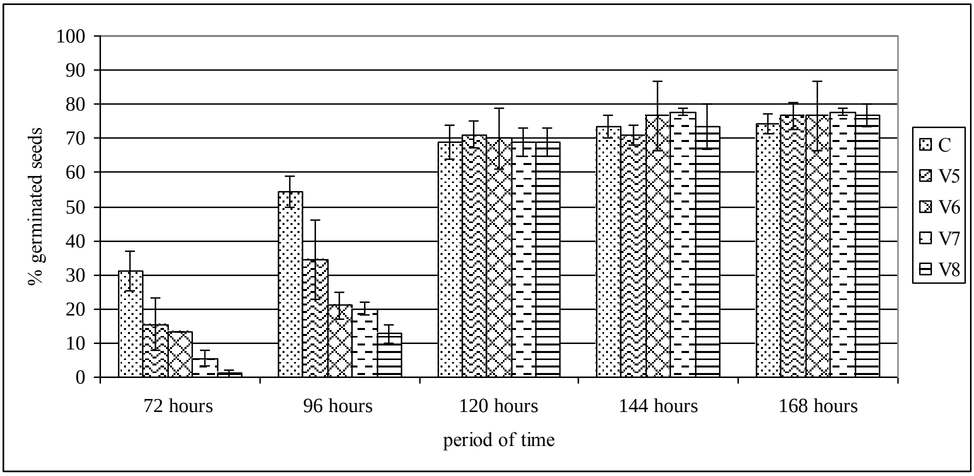

Figure 2. The percentage of the germinated seeds - control and the treatment variants (V5-V8)

Mucilages are non-homogenous polysaccharides; they contain galactans, arabinans, xylans or their associations, which can be accompanied or not by uronic acids. The last ones confer the mucilage an acidic character; the mucilage without uronic acids have a neutral character (Ciulei et al., 1993). Mucilages present high hydrating capacity; they form a gelatinous barrier at the level of the seeds (Windsor et al., 2001). It is considered that the mucilage facilitate the germination and can protect the seeds in relation with other types of organisms (Western et al., 2000). Based on the data in the literature in the field consulted, we consider that mucilage protects the seeds and, by the fact that it maintains the humidity, it allows the germination in stressful conditions (the presence in the substrate of high concentrations of zinc: 400-800 mg/l); it is possible that they represent also a barrier in the entrance of zinc in excess inside the seeds of *Salvia coccinea*. In case of *Ageratum houstonianum* species, the treatment with zinc (zinc sulphate) in a concentration of 400 mg/l, 600 mg/l and 800 mg/l delays the seed germination by 6.93 %, 20.79 % and 23.85 % respectively (96 hours after the beginning of the experiment) (Stratu and Costică, 2013).

The length of the root and of the *hypocotyl.* 168 hours after assembling the experiment, measurements regarding the two parameters studied were done in the control and the variants V1 - V4. We mention the fact that in the case of the treatment with zinc in concentration of 400 mg/l, 500 mg/l, 600 mg/l, 800 mg/l measurements were not made regarding the length of the root and hypocotyl because in these concentrations a pronounced effect of delay, respectively inhibitor on the growth process was noticed. *The length of the root* and *hypocotyls* in the variants of treatment presented average values lower than in the control, values decreasing with the increase in the concentration of the metal (Fig. 3). This fact indicates that the treatment done presents an unfavourable influence on the growth in length of the root and hypocotyls. The delay of the growth in length comparing with the control was more pronounced in the case of the root: by 39.54 % in the variant V2, by 77.22 % in the variant V3 and by 79.23 % in the variant V4. At hypocotyl, the delay in the growth in length comparing with the control was higher in the variants V3 and V4: by 37.84 % and 47.45 % respectively (Fig. 4).

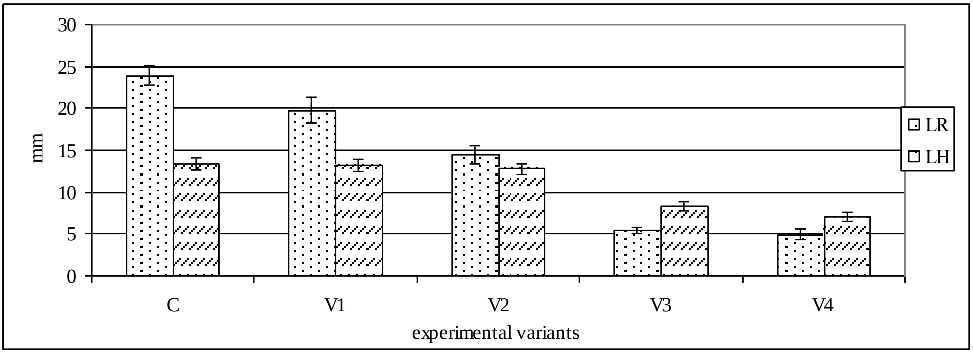

Figure 3. The length of the root (LR) and the hypocotyl (LH)

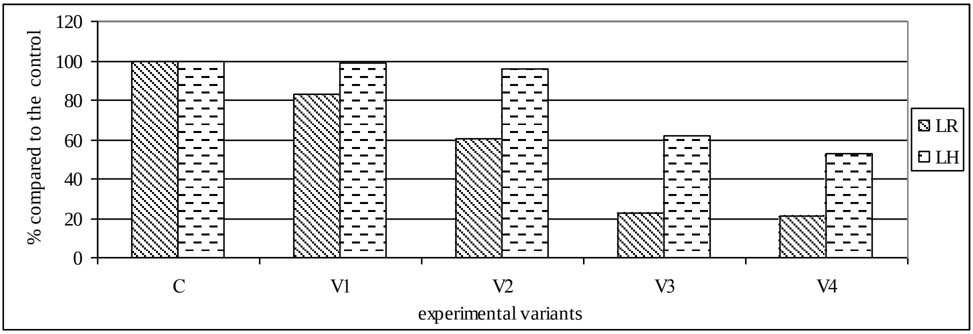

Figure 4. The length of the root (LR) and the hypocotyl (LH) - % compared to the control

The influence is statistically significant; the statistics of Anova unifactorial test (F calculated: 69.44 - length of the root; 22.02 - length of the hypocotyl) was higher than the critical value (2.44 for length of the root and the hypocotyl); $p<0.001$. The results of the Tukey Test indicate the fact that: the control differs significantly from the variants V2, V3, and V4 – for the length of the root; the control differs significantly from the variants V3 and V4 - for the length of the hypocotyl.

Similar effects were recorded in research done by us in the species *Ageratum houstonianum.* The treatment with zinc (zinc sulphate) in a concentration of 400 mg/l, 600 mg/l and 800 mg/l delays the growth of the root and of the hypocotyl (96 hours after the beginning of the experiment); the effect is very pronounced in the case of high concentrations (600 mg/l and 800 mg/l) (Stratu and Costică, 2013).

The results obtained by us confirm some of the data in the literature of specialty. An inhibiting effect for the growth of the root/hypocotyl (by more than 90% compared with the control) caused by high concentration of zinc sulphate (40 mM) were noticed by Nag et al., (1989) in the seedlings of *Vigna radiata* (time of germination 5 days, out of which 72 hours of treatment with solutions of zinc sulphate). Çavuşoğlu et al. (2009) notices at *Phaseolus vulgaris* the significant reduction of the root length after seven days of treatment with zinc in concentration of 30 ppm and 70 ppm. Sharma et al. (2010) indicates the fact that the treatment with zinc (in form of zinc sulphate) in concentration 75 mM and 100 mM reduces significantly the length of the root and of the hypocotyls of the seedlings of *Cicer arietinum* after 15 days of treatment. Also, the delay of growth in length for the root and shoot was reported also by other authors: authors quotes by Khudsar et al. (2004) and Tsonev and Lidon (2012) in the species *Phaseolus mungoo* (Chaoui et al., 1997), *Vigna radiata* and *Sorghum bicolor* (Balashouri, 1995), *Bacopa monniera* (Ali et al., 1999); Shivakumar and Thippeswamy (2012) for *Cicer arietinum*, *Macrotyloma uniflorum*, *Vigna radiata*, *Vigna unguiculata* in the case of concentrations of 100, 200, 500 ppm.

Normally, in conditions of stress, the growth of the root is more affected than the growth of the shoot (Khudsar et al. 2004). The root is the first organ of the plant that comes in contact with the substrate; it absorbs and accumulates metals from the substrate and is considered a true indicator of the tolerance of the heavy metals in plants (Jeliaskova and Craker, 2002 quoted by Shivakumar and Thippeswamy, 2012).

Also, our observations underlined the following aspects: starting with the variant V4 (concentration of 300 mg/l), the root "avoids" the toxic conditions of stress, respectively the contact with the filter paper soaked with solution of zinc; the root is oriented in reverse sense of the substrate or, in some cases, all the seedling is oriented in the opposite direction to the sense of action of the gravitation, the contact with the substrate is done by mucilage (which protects the cotyledon leaves) that maintains the humidity and allows the seedlings to live in conditions of stress; the area at the tip of the root presents a brown colour; the area of growth in length and the process of formation of lateral roots is affected. In the variants V7 and V8, the effect is inhibitor; the root is brownish, the lateral roots are not present, the hypocotyls axis is very short.

The brown colour of the root in some areas is quoted by Vassilev et al. (2011). According to Van Assche F. (1973) quoted by Rout and Das (2003), zinc in high concentrations inhibits the metabolic activity. Woolhouse (1983) synthesising numerous information in the literature in the field, mentions the fact that in many species the toxicity of the zinc is associated with the inhibition of the growth of the root; the process of cell elongation is especially affected. According to Malecka et al., (2012), the inhibition of the growth and the reduction of the biomass are the main responses of the higher plants to the toxicity of heavy metals.

Conclusions

The seeds of *Salvia coccinea* germinate in the presence of some moderate and high concentrations of zinc in substrate.

The concentrations used for the treatment do not influence negatively the germination of the seeds, but affect the growth of the root and hypocotyls. In moderate concentrations, the growth is delayed and in high concentrations the growth is inhibited.

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