THE INFLUENCE OF COPPER ON SOME PHYSIOLOGICAL AND MORPHOLOGICAL INDICATORS OF *IMPATIENS BALSAMINA* L.

INFLUENȚA CUPRULUI ASUPRA UNOR INDICATORI MORFOLOGICI ŞI FIZIOLOGICI LA *IMPATIENS BALSAMINA* L.

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Abstract. *This paper presents the results of a study regarding the influence of treatment with copper in different concentrations (10mg/L, 20mg/L, 40mg/L, 60mg/L, 100mg/L and 150mg/L) on some physiological and morphological indicators of Impatiens balsamina species. The following indicators were analyzed: the percentage of germinated seeds, the mean time of germination, the length of the root and of the hypocotyl, the tolerance index. The following effects were found: the pronounced reduction of the germination percentage and the increase of the germination mean time in high concentration; the delay of the growth in length of the root and the hypocotyl; the decrease of the tolerance index. The root was more affected than the hypocotyl.*
Key words: copper, germination indices, tolerance index

Rezumat. *Lucrarea prezintă rezultatele unui studiu referitor la influența cuprului în concentrații diferite (10mg/L, 20mg/L, 40mg/L, 60mg/L, 100mg/L şi 150mg/L) asupra unor indicatori morfologici şi fiziologici la specia Impatiens balsamina. S-au luat în studiu următori indicatori: procentajul de germinație; timpul mediu de germinație; lungimea rădăcinii şi lungimea hipocotilului; indicele de toleranță. S-au constatat următoarele efecte: reducerea pronunțată a procentajului de germinație şi mărirea timpului mediu de germinație la concentrații mari; reducerea procesului de creştere în lungime a rădăcinii şi a hipocotilului; scăderea indicelui de toleranță. Rădăcina a fost mult mai afectată comparativ cu hipocotilul.*
Cuvinte cheie: cupru, indici de germinație, indice de toleranță

INTRODUCTION

In natural state, copper is found in form of oxygenated compounds (carbonates, oxides, sulphates, silicates) or sulfides (Davidescu *et al.,* 1988). The clay - humic complex from soil has a high affinity absorbtion for copper. The copper solubility in the soil is influenced by several factors: soil organic matter, soil pH, other ions in soil (Caramete *et al.*, 1973). It is absorbed by plants in bivalent ionic form. The copper absorption and their translocation in plants are influenced by several factors: soil pH, the content of the available forms of copper from soil, the level of representation of nitrogen in the soil and plants (Rusu *et al.*, 2005).

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In normal concentration, copper is vital to plant growth and development. Copper has many roles both metabolic and physiological: it is a component of the plastocyanine and numerous enzymes; it activates certain enzymes; it has a role in the processes of oxide reduction, in organogenesis, in the synthesis of the proteins, carbohydrates (Davidescu *et al.*, 1988; Caramete *et al.*, 1973; Maksimiec, 1997; Rusu *et al.*, 2005; Singh *et al*., 2007); stimulates the reproduction of plants (Rusu *et al.,* 2005).

Some human activities such as excessive application of plant protection products, of fertilisers, the mining activities, depositing waste (Śnieg and Nowak, 2005), processing waste (Luo *et al.,* 2011), ferrous ore processing activities contribute to the increase in the content of copper in the soil and the appearance of phenomena of toxicity in plants. According to Kabata - Pendias and Pendias (1993) quoted by Śnieg and Nowak (2005), the maximum content tolerable of copper in the soil is 100mg/kg. In Romania, the maximum permissible limit for the copper content from soil is 20 mg/kg; the alert threshold is 100 mg/kg for the land with sensible use and 250 mg/kg for the land with less sensible use (Ordin nr. 756/1997 pentru aprobarea Reglementării privind evaluarea poluării mediului).

In high concentrations it becomes toxic (Wolhouse, 1983): can reduce/inhibit the germination and the growth of the plants (Ouzounidou, 1995; Kumar *et al.*, 2009; Asharge *et al*., 2013; Gang *et al*., 2013); can affect the photosynthesis, chlorophyll synthesis; caused chlorosis (Wolhouse, 1983; Maksimiec, 1997); the activity of some enzymes is modified (Maksimiec, 1997; Yurekli and Banu Porgali, 2006). Because it is toxic at a certain concentration, copper is included in some pesticides (bactericides, fungicides) used in agriculture (Scheck and Pscheidt, 1998).

Impatiens balsamina L. (Balsaminaceae family) is an annual herbaceous species, decorative (Săvulescu, 1958; Preda, 1989) by its ports (appearance of small tree) and by flowers.The flowers are single and double**,** different colors (white, pink, red, etc) and has a brief spur. It blooms from June and September. It is cultivated in urban green spaces being used in flower arrangements and in private gardens (frequently in the rural areas). It can grow in pots being used to decorate the terraces and the balconies.

It originates in SE Asia (http://www.missouribotanicalgarden.org/). This species can accumulate heavy metals (copper, cadmium) in the root and the leaves (Noorjahan, 2015).

This paper has as purpose the investigation of the copper influence on some physiological and morphological indicators of *Impatiens balsamina* L. species during and after germination phases.

MATERIALS AND METHODS

As biological material, seeds of *Impatiens balsamina* L. have been used and were aquired from a company which merchandises seeds (S. C. Unisem S. A). Copper has been used in the form of copper sulphate solutions. At experiment we used following copper concentrations: 10 mg/L; 20 mg/L; 40 mg/L; 60mg/L; 100 mg/L;

150mg/L. The seeds were disinfected for 6 minutes with solutions of oxygenated water 3 % and afterward washed several times with distilled water. From the test species, 15 seeds were placed, in Petri dishes, on a filter paper. The filter paper was humidified with four ml of distilled water (a control variant) and copper sulphate solutions (treatment variants). The test was done in three replications (three plates) for each experimental variants.The plates were kept in a growth chamber (Snijders Scientific type), at 12:12h photoperiod, 22°C/24°C, and 60% relative humidity for seven days. During the experiment, the substrate of germination was moistened with distilled water and, respectively cooper sulphate solutions. The experiment was completed in March 2016. The influence of cooper was investigated in the germination and post-germination phase taking in the study the following indicators: the percentage of germination, the mean time of germination – calculated by the formula presented in the work published by Moradi *et al*., (2008); the length of the root, the length of the hypocotyls; the tolerance index of heavy metals (TI) - calculated by the formula presented in the work published by Ahmad et al., (2012). The data obtained for the indexes of germination and morphological indicators were stated as average values ± standard errors (n=3 and n= 25); they were statistically interpreted using the unifactorial Anova test and Turkey test (α = 0.05) (Zamfirescu and Zamfirescu, 2008).

RESULTS AND DISCUSSIONS

On the choices of treatment, in comparison with the control, ***the final percentage of germination*** scored a slightly negative value for the readings of V1, V3, V4 and more prominent for readings V5 and V6 (with 34.1% and respectively with 36.38% in comparison with the control), statistically significant ($p<0.05$) (tab. 1).

Table 1

The percentage of germination and the mean time of germination

The variant	The analised indicator (average ± ES)			
	Germination percentage (%)	+/- (%)	Mean time of germination (days)	+/- (%)
Martor	97.77±2.22	0.00	3.35±0.08	0.00
V1(10mg/l)	93.33± 3.85	-4.55	3.19±0.09	-4.78
V2 (20mg/l)	100±0	+2.28	3.15±0.08	-5.98
V3 (40mg/l)	93.33±3.85	- 4.55	3.43±0.12	+2.38
V4 (60mg/l)	93.33±3.85	-4.55	3.13±0.07	-6.57
V5 (100mg/l)	64.44±5.88*	-34.1	4.27±0.21	+27.46
V6 (150mg/l)	62.21±4.44*	-36.38	4.49±0.49*	+34.02

Note: * indicates significant differences (Tukey test, $p<0.05$); +/ - represents the percentage of reduction or increase compared to the control

An exception to the situation presented was recorded in V2 variant (at the concentration of 20mg/L); in this case the germination percentage increase slightly.

The mean time of germination show a pronounced increase compared to the control to V5 and V6 treatment variants; in statistic point of view, the increase was significant ($p<0.05$) only at V6 variant (at the concentration of 150mg/L) (tab. 1). The results of the two indicators show a unfavourable effect of high concentration of copper in the germination process of the test species.

The effects of germination reduction in the case of exposure to various concentrations of copper were reported at: chickpea (Kumar *et al.,* 2009); tomatoes (in concentration of 100ppm and 200ppm) (Asharge *et al.,* 2013); wheat (in concentration of 50ppm - 500ppm) (Gang *et al.,* 2013); wheat (in concentration of 5mg/L, 25mg/L, 50mg/L, 100mg/L) (Singh *et al.*, 2007). In some spontaneous herbaceous species, germination was more affected by high concentrations of copper (80 and 160µM) (Ouzounidou, 1995). At other cultivated species, the exposure to copper determined the stimulation of the percentage of germination: *Phaseolus mungo* (in concentration of 1mM) (Kumar *et al.*, 2009); *Plantago psyllium* (copper sulphate: 40mg/L and 60mg/L) (Mohammadi *et al.,* 2013).

The obtained results about the influence of copper on the germination of *Impatiens balsamina* were due to structural peculiarities of seeds and other factors (properties of the copper ions). According to Araùjo and Monteiro (2005) the tegument of the seed can represent a barrier between the embryo and the environment in the immediate neighbourhood. At the *Impatiens balsamina* the seed tegument is thin.

The length of the root (LR) recorded a significant reduction in value ($p < 0.05$) comparing with the control in V1- V5 variants. At V3-V5 variants, the percentage of the reduction of the root length comparing to the control exceeds 50%, which shows the inhibiting effect of copper on root growth (tab. 2).

Table 2

The morphological indicators and the tolerance index

The variant	The analised indicator (average ±ES)				
	LR (mm)	+/- (%)	LH (mm)	+/- (%)	IT (%)
Martor	46.76±1.48	0.00	12.92±0.47	0.00	-
V1(10mg/L)	33.64±2.41*	- 28.06	12.8±0.66	-0.93	71.94
V2 (20mg/L)	24±2.1*	- 48.68	13.16±0.66	+1.85	51.32
V3 (40mg/L)	15.44±1.45*	- 66.99	12.64±0.7	- 2.17	33.01
V4(60mg/L)	11.64±0.81*	-75.11	10.96±0.56	-15.18	24.89
V5 (100mg/L)	7.56±0.62*	- 83.84	9.24±0.53*	- 28.49	16.16
V6 (150mg/L)	-	-	8.44±0.45*	- 34.68	-

Note: * indicates significant differences (Tukey test, $p<0.05$); +/ - represents the percentage of reduction or increase compared to the control

The lenght of the hypocotyl (LH) recorded a decrease compared to the control (except V2 variant), stastically significant ($p< 0.05$) only at V5 and V6 variants. These results indicate a negative influence on root and hypocotyl elongation, root was more affected than hypocotyl (tab. 2).

The percentage of the reduction of the root and hypocotyl elongation comparing with the control increases with the increase of the concentration of the solution: negative, strong correlation (r = - 0.886) for the length of the root and very strong (r = - 0.965) for the length of the hypocotyl.

The reduction of the length growth of the root, and respectively of the root and hypocotyl/seedlings was reported by other authors, too: Yurekli and Banu Porgali (2006) at the bean root; Kumar *et al*. (2009) at the chickpea and bean; Ashagre *et al*. (2013) at tomatoes; Gang *et al*. (2013) at the wheat root; Singh et al. (2007) at the wheat coleoptyle and root. Ouzounidou (1995) found that the Cu^{2+} ions in high concentration reduced the growth of the primare root at *Alyssum montanum* and *Thlaspi ochroleucum.*

The copper ions in excess inhibit the elongation, inducing the formation of free radicals that cause oxidative stress and the alterations at the cellular level (affect the permeability of the membranes and the proteins synthesis, increase the rate of peroxidation of the lipids) (Maksimiec, 1997; Yurekli and Banu Porgali, 2006; Singh *et al.*, 2007). According to Cheng (2003), the stress caused by heavy metals reduces the vitality of the root and affects the process of growth.

The tolerance index (TI) presented a gradual decrease in value, with the increase of the metal concentration. At high concentrations of copper used was low tolerance (tab. 2). Low tolerance can be caused by alterations of some physiological and biochemical processes. A low tolerance to high concentrations of copper was reported at: *Cicer arietinum* and *Phaseolus mungo* (Kumar *et al.*, 2009); *Lycopersicon esculentum* (Asharge *et al.,* 2013).

CONCLUSIONS

1. The seeds of *Impatiens balsamina* germinated in the presence of some moderate and high concentrations of copper in substrate. The process of germination was significantly reduced only at high concentrations.

2. The root and hypocotyls elongation was negatively influenced by all concentrations used in the treatment; the root was more affected than the hypocotyl.

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