Copper Toxicity on *Triticum aestivum* L and *Lactuca sativa* L: Effects on Germination and Growth

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ABSTRACT

Copper is necessary for the growth and development of plants, but at high concentrations it is extremely toxic, resulting in growth inhibition and toxicity symptoms. In this study we investigated the effects of seed immersion on *Triticum aestivum* L and *Lactuca sativa* L for 24 hours, at different concentrations of $\text{CuSO}_4 \times 5\text{H}_2\text{O}$ (0.05g/L - variant V1; 0.1g/L - variant V2; 0.5 g/L - variant V3; 1 g/L - variant V4). There were investigated the seed germination, root and shoot lengths, tolerance index and phytotoxicity index. The result of metabolic disturbances induced by the treatment of vegetative organs was their growth inhibition in a dose-dependent manner.

Keywords: copper, seed germination, index of phytotoxicity, root length, shoot length.

INTRODUCTION

Cooper is an important microelement for plants. It activates certain enzymes (catalase, hydrogenase, cytochrome oxidase), it stimulates the formation of chlorophyll, intervenes in the metabolism of carbohydrate and oxidative processes, it stimulates the nitrogen fixation and seed germination (Yruela, 2005, 2009).

Copper sulphate is used as an antifungal agent in many pesticides (Almeida *et al.*, 1998). Long-term applied fungicides allow the accumulation of copper in soil. Copper, once in soil, attaches itself to organic materials in the upper layers of soil. That is why it does not migrate over long distances in soil. This type of metal pollution is a problem caused by the penetration of metal into the structure of the food chains. In high concentrations in soil, it affects plant tissues and it can cause deficiencies in other essential nutrients due to antagonistic interaction (Kabata-Pendias and Pendias, 2001).

The excess of copper affects the activity of enzymes, it impairs the DNA, the protein oxidation and the integrity of membranes which alters the photosynthesis, and it damages plasma

membranes and produces functional changes and other metabolic disorders (Lou *et al.* 2004; Azooz *et al.*, 2012; Ye *et al.* 2014).

The aim of this study was assessing the effects of the immersion on *Triticum aestivum* caryopsis and *Lactuca sativa* seeds into different concentrations of copper sulphate, namely: seed germination, root and shoot lengths, tolerance index and phytotoxicity index.

MATERIALS AND METHODS

The experiments have been carried out on two species *Triticum aestivum* L Mara and *Lactuca sativa* L. May King. *Lactuca sativa* has been used as a test organism due to its sensitivity to chemical agents, high rate of germination and rapid growth. *Lactuca sativa* var. *capitata* L. May King is a garden seed line product from Hungary. The caryopsis of *Triticum aestivum* L Mara is a Green master product.

Germination vessels were Linhardt dishes. The conditions of the experiment were the following: temperature of 22^o C, relative air humidity of 38.30% and photoperiod from 12 to

13 hours. During the experiment, germination substrate was distilled water. For each variant, we used two replications (each with 30 seeds).

The phytotoxicity test followed the effects of immersion on seed with $\text{CuSO}_4 \times 5\text{H}_2\text{O}$ concentrations (0.05 g/L – variant experimental V1; 0.1g/L – variant experimental V2; 0.5 g/L – variant experimental V3; 1 g/L – variant experimental V4). The standard evaluation of ecotoxicity is presented in the following procedures ISTA 1999.

The seed germination capacity was assessed by two indices: germination energy and germination faculty. Readings on every four day were made to determine the germination energy and on every seven day (*Triticum aestivum*) or ten day (*Lactuca sativa*) for the germination faculty. The vegetative organs (root and shoot) were measured on the fourteen day.

The relative increase in length of root, the tolerance index and the percentage of phytotoxicity of root and phytotoxicity of shoot were determined.

The relative increase in length of root, the tolerance index and the percentage of phytotoxicity

of root and shoot were determined (Baber and Walker, 1989). Index of tolerance was determined using equations 1 (Shaw, 1989). The percentage of phytotoxicity on shoot and root was calculated following the formula 2 (Chou and Lin, 1976).

After fourteen days, the root and shoot was separated. Initially, it was determined the fresh mass of vegetative organs and then the dry weight.

Statistical processing was determined through different mathematical methods. In some cases, were calculated percent, and in other cases the data obtained has been processed statistically by the Student's "T" test. The aberrant values have been eliminated according to the Chauvenet criterion (Snedecor and Cochran 1978; Weber, 1980). The statistic consideration has been considered from $p < 0.05$.

RESULTS AND DISCUSSION

The process of germination was determined by means of the indicators that are considered representative in the specialty literature.

Triticum aestivum caryopsis germinated the first day (Tab. 1). The germination percentage was between 85% (control variant-R1) and 0%

$$T.I. = (\text{Mean root length in metal solution} / \text{Mean root length in distilled water}) \times 100 \quad (1)$$

$$\text{Phytotoxicity } \oint \text{ shoot } (\%) = \frac{\text{Shoot length } \oint \text{ control} - \text{Shoot length } \oint \text{ treatment}}{\text{Shoot length } \oint \text{ control}} \times 100 \quad (2)$$

$$\text{Phytotoxicity } \oint \text{ root } (\%) = \frac{\text{Shoot length } \oint \text{ control} - \text{Shoot length } \oint \text{ treatment}}{\text{Shoot length } \oint \text{ control}} \times 100$$

Tab. 1. Germinative energy and germinative faculty for *Triticum aestivum*

	M		V1		V2		V3		V4	
R1/R2 Day	R. 1 %	R. 2 %	R. 1 %	R. 2 %	R. 1 %	R. 2 %	R. 1 %	R. 2 %	R. 1 %	R. 2 %
1 day	85	65	95	80	80	100	5	0	0	0
2 days	100	90	95	95	90	100	65	35	50	25
3 days	100	90	95	95	95	100	90	85	70	45
4 days	100	90	95	95	95	100	90	85	80	55
5 days	95	90	100	100	95	100	95	90	80	60
6 days	95	90	95	95	95	100	95	90	80	60
7 days	95	90	95	95	95	100	90	90	75	55

Note: M = control variant; V1 = experimental variant (0.05 g/L); V2 = experimental variant (0.1g/L); V3 = experimental variant (0.5 g/L); V4 = experimental variant (1 g/L). R.1 – first replication. R. 2 – second replication.

Fig. 1. Seedlings of *Triticum aestivum* (after seven days)

(V4 experimental variant – R1). The germinative energy was calculated after four days. Germinative energy of seed germination expresses speed in the terms of the experiment. For *Triticum aestivum*, it had values between 100% (control variant - R1), 95% (V1 and V2 experimental variants – R1), 85% (V3 experimental variant – R1) and 80% (V4 experimental variant - R1).

The germination energy values ranged from 90% (control), 95% (V1 experimental variant), 100% (V2 experimental variant), 85% (V3

experimental variant) to 55% (V4 experimental variant) in the second replication.

The germinative energy values ranged from 90% (control variant), 95% (V1 experimental variant), 100% (V2 experimental variant), 90% (V3 experimental variant) to 55% (V4 experimental variant) in the second replication.

Germinative faculty was determined in the seventh day of the experiment (Tab. 1, Fig. 1). In case of *Triticum aestivum*, first replication, the germinative faculty was between 95% (M, V1, V2

Tab. 2. Germinative energy and germinative faculty for *Lactuca sativa*

	M		V1		V2		V3		V4	
R1/R2	R. 1	R. 2	R. 1	R. 2	R. 1	R. 2	R. 1	R. 2	R. 1	R. 2
Day	%	%	%	%	%	%	%	%	%	%
1 day	90	85	35	60	10	5	0	0	0	0
2 days	90	90	75	90	80	75	35	30	20	15
3 days	95	90	85	95	90	95	70	75	65	65
4 days	100	90	85	95	90	95	100	100	100	65
5 days	100	90	100	100	95	95	100	100	100	100
6 days	100	90	100	100	95	95	100	100	100	100
7 days	100	90	100	95	95	95	100	100	100	100

Explication at table 1.

Fig. 2. Seedlings of *Lactuca sativa* (after seven days)

experimental variants) 90% (V3 experimental variant) and 75% (V4 experimental variant). The germinative faculty ranged from 100% (V2 experimental variant), 95% (V1 and M experimental variants), 90% (V3 experimental variant) to 55 % (V4 experimental variant) in the second replication.

The seeds of *Lactuca sativa* germinated immediately (Tab. 2, Fig. 2). In the first day, the germinated percent was between 90% (control variant - R1) and 0% (V3 and V4 experimental variant - R 1). The seed germination speed for *Lactuca sativa* was between 100% (M,

V3, V4 experimental variants - R1), 85% (V1 experimental variant - R1) and 90% (variant V2 -R1). Germinative faculty for *Lactuca sativa* was determined in the 10th day of the experiment. Germinative faculty was between 100% (M, V1, V3, V4 experimental variants - R1) and 95% (V2 experimental variant). Similar results were obtained in the second replication.

The germination of caryopsis is influenced by the immersion on seeds into various concentrations of copper sulphate. Caryopsis immersed into concentrations of 0.5g/l and 1 g/l of copper sulphate showed low levels of energy

Tab. 3. Germination index of *Triticum aestivum* and *Lactuca sativa*

Exp. variants	<i>Triticum aestivum</i>		<i>Lactuca sativa</i>	
	Replication 1 (%)	Replication 2 (%)	Replication 1 (%)	Replication 2 (%)
V1	100.00	105.55	100.00	105.55
V2	100.00	111.11	95.00	105.55
V3	94.73	100.00	100.00	111.11
V4	78.94	61.11	100.00	100.00

Explication in Tab. 1.

and germination. Lu *et al.* (1998) found that alpha-amylase is important during the late phase of the germination. The deficiencies that occur during the caryopsis germination are due to the initial treatment of the caryopsis.

Table 3 shows the data referring to the germination index. The germination index of *Triticum aestivum* ranges between 100% (V1 experimental variant) and 78.94% (V4 experimental variant), in the first replication. The values of the germination index ranged between 105.55% (V1 experimental variant) and 61.11% (V4 experimental variant) in the second replication.

The germination index of *Lactuca sativa* ranged between 100% and 95.00% in the first replication, and 100% (V4 experimental variant) and 111.11% (V3 experimental variant) in the second replication.

Table 4 presents medium values and standard errors, calculated after the measurement of vegetative organs (root and shoot) of *Triticum aestivum*. The roots from control plants had 10.18 ± 0.66 mm. In case of experimental variants V1 and V2, after 14 days of treatment with copper sulphate, average values reached 13.18 ± 1.27 mm (+29.46%) or 14.98 ± 0.78 mm (+47.15%). The treatment with a concentration of 0.5 g/l and 1 g/l copper sulfate determined a low growth of the root. Percentage differences from control decreased with 8.55% or 56.98%. The immersion

of caryopsis into solutions 0.5g/l and 1 g/l strongly influenced the roots elongation. Information found in the specialty literature (Bravin *et al.*, 2010; Gajewska and Skladowska, 2010) mentions that, for many species, the copper toxicity is associated with the inhibition of the root elongation of seedling.

Regarding shoots, percentage differences from control values indicated a decrease with 49.81% and 51.46% in the experimental variants V3 and V4.

Table 4 shows the results obtained in the second replication. The immersion of wheat caryopsis into concentrated solutions of 0.5g/l and 1 g/l had an inhibitory effect on the seedlings growing and development.

Many studies (Krzeszowska, 2011; Truță *et al.*, 2013) have exposed that copper accumulates in the root, the apoplastic compartment is the head quarters of this accumulation, which causes the hardening of the cellular wall and the inhibition of the root elongation.

The results obtained after fourteen days of treatment with copper sulphate to *Lactuca sativa* are presented in tables 5. *Lactuca sativa* is sensitive to the stress produced by various concentrations of copper sulphate. Delays in root growth compared to the control were observed in all experimental variants.

The diminishing compared to the control was between -24.24% in the V1 experimental variant

Tab. 4. The influence of copper sulphate treatment, after fourteen days, on *Triticum aestivum* caryopses, first replication (R1) and second replication (R2)

Exp. variants	Root length (mm)			Shoot length (cm)		
	X ± ES	D%	P	X ± ES	D%	p
M – R1	10.18 ± 0.66	-	<0.05	5.46 ± 0.20	-	<0.05
M – R2	11.30 ± 0.78	-	<0.05	5.02 ± 0.31	-	NS
V1 – R1	13.18 ± 1.27	+29.46	NS	5.26 ± 0.19	-3.66	<0.05
V1 – R2	14.13 ± 0.78	+25.04	<0.01	5.52 ± 0.26	+9.96	<0.05
V2 – R1	14.98 ± 0.78	+47.15	<0.01	5.02 ± 0.28	-8.05	<0.05
V2 – R2	14.61 ± 1.48	+25.30	NS	4.74 ± 0.37	-5.57	NS
V3 – R1	9.31 ± 0.06	-8.55	<0.01	2.74 ± 0.35	-49.81	NS
V3 – R2	6.65 ± 1.65	-41.16	NS	2.74 ± 0.43	-45.41	NS
V4 – R1	4.38 ± 1.96	-56.98	NS	2.65 ± 0.48	51.46	NS
V4 – R2	6.23 ± 1.14	-44.87	NS	1.56 ± 0.11	68.92	NS

Note: The table includes the average ± the standard error (X ± ES), percentage differences as compared to the control (D%) and statistic significance considered from p<0.05. The statistically non-significant values are marked with NS.

Tab. 5. The influence of the treatment with copper sulphate after fourteen days on *Lactuca sativa* seedlings, first replication

Exp. variants	Root length (mm)			Shoot length (cm)		
	X ± ES	D%	P	X ± ES	D%	p
M – R1	1.98 ± 0.20	-	NS	2.23 ± 0.22	-	NS
M – R2	2.74 ± 0.25	-	NS	2.22 ± 0.16	-	NS
V1 – R1	1.50 ± 0.22	-24.24	NS	1.31 ± 0.20	-41.25	<0.05
V1 – R2	2.00 ± 0.23	-27.10	<0.01	1.31 ± 0.12	-40.99	<0.01
V2 – R1	1.13 ± 0.16	-42.92	<0.05	0.73 ± 0.06	-67.27	<0.05
V2 – R2	2.42 ± 0.20	-11.68	NS	0.55 ± 0.05	-75.22	<0.05
V3 – R1	0.74 ± 0.13	-62.62	<0.01	0.28 ± 0.03	-87.44	NS
V3 – R2	0.54 ± 0.15	-80.30	NS	0.45 ± 0.05	66.21	NS
V4 – R1	0.14 ± 0.01	-92.92	NS	0.21 ± 0.01	-90.58	NS
V4 – R2	0.39 ± 0.03	-85.77	NS	0.75 ± 0.34	-79.72	<0.05

Explication at table 4.

Tab. 6. The mass of the vegetative organs and their physiological reports on *Triticum aestivum* (first replication)

Exp. variants	Vegetative mass (g)					Physiological reports				
	Root		Shoot		DW/DF	Root		DW/FW	Shoot	
	FW	DW	FW	DW		%DW	C%		%DW	C%
M	0.014	0.010	0.083	0.075	0.714	71.40	100	0.903	90.30	100
V1	0.009	0.006	0.035	0.029	0.666	66.60	93.28	0.828	82.80	91.69
V2	0.015	0.013	0.039	0.035	0.866	86.66	121.37	0.897	89.70	99.34
V3	0.002	0.001	0.021	0.018	0.500	50.00	70.02	0.857	85.70	94.91
V4	0.002	0.0002	0.014	0.010	0.100	10.00	14.01	0.714	71.40	79.07

Note: FW – fresh weight; DW – dry weight. M = control variant; V1 = experimental variant (0.05 g/L); V2 = experimental variant (0.1g/L); V3 = experimental variant (0.5 g/L); V4 = experimental variant (1 g/L). R 1 – first replication. R. 2 – second replication.

Tab. 7. The mass of the vegetative organs and their physiological reports on *Triticum aestivum* (second replication)

Exp. variants	Vegetative mass (g)					Physiological reports				
	Root		Shoot		DW/DF	Root		DW/FW	Shoot	
	FW	DW	FW	DW		%DW	C%		%DW	C%
M	0.028	0.019	0.092	0.088	0.956	95.60	100	0.957	95.70	100
V1	0.0095	0.0007	0.035	0.034	0.971	97.10	101.6	0.974	97.40	101.78
V2	0.0058	0.004	0.020	0.015	0.750	75.00	78.45	0.750	75.00	78.38
V3	0.004	0.037	0.023	0.018	0.782	78.20	81.80	0.783	78.30	81.81
V4	-	-	0.026	0.020	0.769	76.90	80.44	0.769	76.90	80.35

Explication at table 6.

and 92.92% in the V4 experimental variant, in the first replication. The growth of the shoot was also affected. The decrease compared to the control was between 41.25% (V1 experimental variant) and 90.58% (V4 experimental variant). The obtained results indicate the reduction of vegetative organs, in the second replication.

The relative growth index in length of root was reduced with 8.55% compared with the control in the V3 experimental variant and with 56.97% in the V4 experimental variant, in the first replication. The relative growth index in length of root was reduced with 41.15% compared to the control in the V3 experimental variant and with 44.87% in the V4 experimental variant, in the second replication. The studies performed by Bravin *et al.* (2010), Gajewska and Skadowska (2010) underlined that the inhibition of root elongation would be due to the inhibition of cellular division.

The relative growth index in length of root on *Lactuca sativa* was reduced compared to the control between 24.25% (V2 experimental variant) and 92.83% (V4 experimental variant) in first replication and in the second replication the reductions were between 12% (V2 experimental variant) and 85.82% (V4 experimental variant).

The weight of the vegetative organs shows the intensity of the growing and development processes, thus it is an indicator of the plant physiological processes. The experiments performed on wheat underlined the vegetal mass decrease of *Triticum aestivum* (Tab. 6 and 7). The decrease of fresh root mass was performed from 0.014 g (control variant) to 0.002 g (V4 experimental variant), in the first replication,

and from 0.028 g (control variant) to 0.004 g (V3 experimental variant), in the second replication.

Another studied parameter was the ratio between the dried and fresh mass. The ratio of dried mass/fresh mass was 0.714 (control variant) and 0.100 (V4 experimental variant), for roots. The ratio of dried mass/fresh mass was 0.903 (control variant) and 0.714 (V4 experimental variant), in case of shoots.

Other physiological parameters taken into account were the amount of dry mass of 100 g fresh. In the first replication, this parameter decreases from 71.40 g% (control variant) to 10 g% (V4 experimental variant) in the case of the roots. The same results were also observed in the second replication. The percentage of dried substance decreased from 95.60 g% (control) to 76.90 g% (V4 experimental variant).

In case of the shoot dry mass quantity from 100 g fresh mass decreases from 90.30 g% (control variant) to 71.40 g% (V4 experimental variant) in the first replication. The percentage of 100 g dried substance decreased from 95.70 g% (control variant) to 76.90 g% (V4 experimental variant) in the second replication. The immersion of wheat caryopsis influenced the further growth and development of vegetative organs. The inhibition of vegetative organs is associated with the decrease of fresh mass and further dried mass.

Similar results were also observed for *Lactuca sativa* (Tab. 8 and 9). Physiological changes, that have affected the growth and development of vegetative organs, were produced, as the concentration of metal in the solution, into which the seeds were immersed, increased. The

Tab. 8. The mass of the vegetative organs and their physiological report on *Lactuca sativa* (first replication)

Exp. variants	Vegetative mass (g)					Physiological reports				
	Root		Shoot			Root		Shoot		
	FW	DW	FW	DW	DW/DF	%DW	C%	DW/FW	%DW	C%
M	0.668	0.543	0.412	0.355	0.813	81.30	100	0.862	86.20	100
V1	0.289	0.168	0.107	0.004	0.581	58.10	71.46	0.037	3.70	4.29
V2	0.563	0.442	0.412	0.345	0.788	78.80	96.92	0.837	83.70	97.10
V3	0.084	0.042	0.216	0.163	0.500	50.00	61.50	0.756	75.60	87.70
V4	0.050	0.026	0.130	0.088	0.520	52.00	6.96	0.677	67.70	78.54

Explication at table 6.

Tab. 9. The mass of the vegetative organs and their physiological report on *Lactuca sativa* (second replication)

Exp. variants	Vegetative mass (g)					Physiological reports				
	Root		Shoot		DW/ FW	Roots		DW/ FW	Shoot	
	FW	DW	FW	DW		%DW	C%		%DW	C%
M	0.782	0.650	0.452	0.387	0.831	83.10	100	0.856	85.60	100
V1	0.139	0.058	0.126	0.075	0.417	41.70	50.18	0.595	59.50	69.51
V2	0.620	0.473	0.393	0.328	0.763	76.30	91.82	0.835	83.50	97.51
V3	0.070	0.043	0.171	0.123	0.614	61.40	73.89	0.719	71.90	84.00
V4	0.011	0.003	0.062	0.036	0.273	27.30	32.85	0.581	58.10	67.87

Explication at table 6.

inhibition of growth decreases the vegetative mass of the superior plants study.

The tolerance index for *Triticum aestivum*, decreased in the first replication from 129.47% (V1 – experimental variant) to 33.23 % (V4 – experimental variant), and for the species *Lactuca sativa* decreased from 75.76% (V1 – experimental variant) to 7.07% (V4 – experimental variant).

The tolerance index of the species decreased as the concentration of copper sulphate increased, into which the seeds were immersed. The non-tolerant plants showed decreased the growth while the tolerant ones were stimulated by the elevated copper concentrations for optimal growth.

Phytotoxicity of shoot and root increased as the concentration of copper sulphate increased. The highest copper phytotoxicity on root (56.97%) and shoot (51.47%) were observed at 1 g/l, in first replication. For the second replication, phytotoxicity on root and shoot had the following values 44.87% and 68.92%.

The results of this study were according to the studies made by Shaikh *et al.* (2013). The negative effects of copper sulphate on shoot and root lengths, have a direct relationship to their toxicity on shoots and roots on species studied.

CONCLUSION

The immersion of *Triticum aestivum* caryopsis and of *Lactuca sativa* seeds in various concentrations of copper sulphate (0.05 g/L – experimental variant V1; 0.1g/L – experimental variant V2; 0.5 g/L – experimental variant V3; 1 g/L – experimental variant V4) has determined the following effects. The diminishing of the

germination capability in both species it is according to the metal concentration of the solution used for immersion.

The stress produced by the metal solution has determined a strong inhibition in the root elongation. The root reduced growth has affected the shoot growth too, as the root plays a role in the transportation of small quantities of nutrients and of water up to plant aerial parts. The modification of the length in both the shoot and the root, has determined the diminishing of the fresh and dry vegetal mass.

As we have gradually increased the concentration of metal solution in which the seeds were immersed, there have appeared physiological modifications that have affected the vegetative organs. The effects of the copper sulphate on the vegetative organs depend on the copper sulphate concentration.

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