

THE INFLUENCE OF COPPER ON SEED GERMINATION AND GROWTH IN THE FIRST ONTOGENETIC STAGES IN THE SPECIES *BRASSICA OLERACEA* L. AND *CUCURBITA PEPO* L.

INFLUENȚA CUPRULUI ASUPRA GERMINAȚIEI SEMINTELOR ȘI A CREȘTERII ÎN PRIMELE STADII ONTOGENETICE LA SPECIILE *BRASSICA OLERACEA* L. ȘI *CUCURBITA PEPO* L.

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Abstract. This paper presents the results of a study regarding the influence of treatment with copper in different concentrations (60mg/l, 100mg/l and 150mg/l) on germination and incipient growth on two crop 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 chlorophyll fluorescence. In both species, the following effects were recorded: a statistically not significant increase in germination percentage; a reduction of the germination mean time, a negative influence on root and hypocotyle elongation; a reduction of the tolerance index, a reduction of the chlorophyll fluorescence $\Phi PSII$ parameter. *Brassica oleracea* was more sensible compared to *Cucurbita pepo* towards the applied treatments.

Key words: copper, chlorophyll fluorescence, germination indices

Rezumat. Lucrarea prezintă rezultatele unui studiu referitor la influența cuprului în concentrații diferite (60mg/l, 100mg/l și 150mg/l) asupra germinației și creșterii incipiente la două specii cultivate. S-au luat în studiu următorii indicatori: procentajul de germinație; timpul mediu de germinație; lungimea rădăcinii și lungimea hipocotilului; indicele de toleranță și fluorescența clorofilei. S-au constatat următoarele efecte la ambele specii test: creștere valorică nesemnificativă a procentajului de germinație; reducerea timpului mediu de germinație, influență negativă asupra elongației rădăcinii și a hipocotilului; scăderea indicelui de toleranță, reducere valorică a parametrului fluorescenței clorofilei $\Phi PSII$. *Brassica oleracea* comparativ cu specia *Cucurbita pepo* a fost mai sensibilă la concentrațiile aplicate.

Cuvinte cheie: cupru, fluorescența clorofilei, indici de germinație

INTRODUCTION

Copper is a microelement with multiple physiological roles: it is a component of the plastocyanine and numerous enzymes (cytochrome c oxidase, superoxide dismutase etc.); it activates certain enzymes; it has a role in the processes of oxide reduction, respiration, photosynthesis, in the synthesis of the chlorophyll, on organogenesis, in the synthesis of the proteins, carbohydrates

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(Davidescu *et al.*, 1988; Maksimiec, 1997; Rusu *et al.*, 2005).

In natural state, copper is found in form of oxygenated compounds or sulfides (Davidescu *et al.*, 1988). It is absorbed by plants in bivalent ionic form. The copper solubility in the soil, its absorption and translocation in plants are influenced by several factors (Rusu *et al.*, 2005). Industrial emissions, applying in excess plant products, fertilisers, mining activities, depositing waste (Śnieg and Nowak, 2005), processing waste (Luo *et al.*, 2011) 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.

Even though copper is essential for the life of plants, in high concentrations it becomes toxic (Wolhouse, 1983). As manifestations of copper excess, are quoted: reduction/inhibition of the germination and the growth of the root/growth of seedlings/plants (Kumar *et al.*, 2009; Asharge *et al.*, 2013); chlorosis, it is affected the permeability of the membranes, chlorophyll synthesis, photosynthesis (Wolhouse, 1983; Maksimiec, 1997); the activity of some enzymes is modified (Maksimiec, 1997; Yurekli and Banu Porgali, 2006).

Brassica oleracea L. var. *capitata* is a valuable food plant, with therapeutic properties and average economic and apicultural share (Pârvu C., 2005). This species can accumulate heavy metals (copper, zinc, lead etc.) in the root and aerial organs (Luo *et al.*, 2011; Rădulescu *et al.*, 2013). *Cucurbita pepo* presents alimentary and fodder value, medicinal properties and average economic and apicultural share (Pârvu, 2003); it can accumulate organic compounds and heavy metals in root and in leaves (Mattina *et al.*, 2003). The species that accumulate large quantities of heavy metals could be used to remediate the polluted soils.

This paper has as purpose the investigation of the copper influence on the germination and growth of seedlings in the species *oleracea* L. var. *capitata* and *Cucurbita pepo* L. var. *oblonga* Wild.

MATERIAL AND METHOD

The biological material, was represented by seeds of *Brassica oleracea* L. var. *capitata* - "Dittmarcher" and *Cucurbita pepo* L. var. *oblonga* Wild - "Crișan" obtained from S. C. Unisem S. A. Iași. The experimental variants consisted in: control variant (with distilled water) and three variants of treatment with solutions of copper sulphate (copper concentrations: 60 mg/l; 100 mg/l; 150 mg/l). In selecting the concentrations used for the experiment we started from the critical concentration of copper in soil (60 mg/l) (according to Alloway, 1990; Beckett and Davis, 1979, quoted by www.cprm.gov.br /). The seeds were disinfected for 12 minutes at the pumpkin and 8 minutes at the white cabbage, with solutions of oxygenated water 2 % 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 copper sulphate solutions (treatment variants). The plates were kept at room temperature (20 - 23 °C), a photoperiod corresponding to the month of May, 2015. The total duration of the experiment was of 9 day after mounting. For each variant we used four replications, each replication with ten seeds at pumpkin and 20 seeds at white cabbage. Germinated seeds were counted each 24 hours.

The following indicators were analyzed: the percentage of germinated seeds (at 8 days for the pumpkin and 9 days for the white cabbage); the mean time of germination; the length of the root, the length of the hypocotyl; the tolerance index; the chlorophyll fluorescence. The mean time of germination was calculated by the formula described by Ellis and Roberts, (1981) (Moradi *et al.*, 2008). The tolerance index of heavy metals (TI) was calculated by the formula described by Iqbal and Rahmati, (1992) (Ahmad *et al.*, 2012). The fluorescence of the chlorophyll was measured at cotyledon leaves at pumpkin (three cotyledon leaves from each variant of the experiment), by using a portable fluorimeter for chlorophyll of type Hansatech Instruments FMS2 (England). The fluorescence parameters measured were: Fs (state of fluorescence balance) measured by applying on the surface of the leaf of a luminous modulator flux of low intensity, in an interval of 1.8 seconds; Fm' (maximum intensity of the fluorescence emitted by the tissue adapted to light), by applying for 0.7 seconds of a saturating pulse with an intensity $> 3000 \mu\text{mol}/\text{m}^2/\text{s}$; the quantum efficiency of the photo system II (ΦPSII) as being the proportion $((\text{Fm}' - \text{Fs}) / \text{Fm}')$.

The results presented in tables are expressed as mean value \pm standard error (for the germination percentage and the mean germination time $n = 4$; for the morphological indicators $n = 20$). The data obtained from the germination indices, and the morphological indicators and the fluorescence parameter ΦPSII were statistically interpreted. The unifactorial Anova test followed by the Tukey test ($\alpha = 0.05$) was used in order to test the differences between means (Zamfirescu and Zamfirescu, 2008).

RESULTS AND DISCUSSIONS

In the treatment variants, comparing with the control, ***the final percentage of germination*** recorded a slight increase in value, but insignificant statistically ($p < 0.05$) (Table 1). An exception to the current situation was registered at the pumpkin: at the concentration of 60 mg/l, the percentage of germination was reduced insignificantly from the statistic point of view (by 10.72%) compared to the control.

The mean time of germination was reduced in all the treatment variants (with the exception of the variant V3 - at the pumpkin), comparing with the control, the reduction being statistically significant ($p < 0.05$) only at the white cabbage, at the concentration of 150 mg/l (Table 1).

The effects of germination stimulation in the case of exposure to various concentrations of copper were reported at: *Phaseolus mungo* (in concentration of 1 mM) (Kumar *et al.*, 2009); psyllium (copper sulphate: 40 mg/l and 60 mg/l) (Mohammadi *et al.*, 2013). At other cultivated species, the exposure to copper determined the reduction of the percentage of germination: chickpea (Kumar *et al.*, 2009); tomatoes, cultivar Roma VF, (in concentration of 100 ppm and 200 ppm) (Asharge *et al.*, 2013); wheat, variant GW-366 (in concentration of 50 ppm - 500 ppm) (Gang *et al.*, 2013). It is considered that the tegument of the seed can represent a barrier between the embryo and the environment in the immediate neighbourhood (Araújo and Monteiro, 2005). The morphological and structural particularities of the seminal tegument in the two species can influence the penetration of the ions of copper. At the pumpkin, the seed tegument is sclerified and at cabbage it is thin.

Table 1

The percentage of germination and the mean time of germination

Species	The variant	The analysed indicator			
		Germination percentage (%)	+/- (%)	Mean time of germination (day)	+/- (%)
<i>Brassica oleracea</i>	Control	82,5±3,22	0.00	3,65±0,27	0.00
	V1(60mg/l)	91,25±1,25	10,6	3,16±0,10	-13,43
	V2 (100mg/l)	90±3,53	9,09	3,19±1,14	-12,61
	V3 (150mg/l)	91,25±2,39	10,6	3,05±0,12*	-16,44
<i>Cucurbita pepo</i>	Control	70±4,08	0.00	3,78±0,07	0.00
	V1(60mg/l)	62,5±4,78	-10,72	3,40±0,10	-10,06
	V2 (100mg/l)	85±5	21,42	3,68±0,15	-2,65
	V3 (150mg/l)	72,5±2,5	3,75	3,81±0,25	0,79

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

The length of the root (LR) and of the **hypocotyls (LH)** recorded a significant reduction in value ($p < 0.05$) comparing with the control in both species (with the exception of the variant V1 – white cabbage, hypocotyl). This fact indicates a negative influence on the elongation of the root and hypocotyl (Table 2). The percentage of the reduction of the root elongation comparing with the control increases with the increase of the concentration of the solution (negative, strong correlation; $r = -0.906$ for the length of the root at white cabbage and $r = -0.986$ for the length of the root at pumpkin), fact that indicated a high toxicity of the copper on the root of the two test species. This fact was also presented by Kumar *et al.*, (2009) at chickpea and *Phaseolus mungo*.

Table 2

The morphological indicators and the tolerance index

Species	The variant	The analysed indicator				
		LR (mm)	+/- (%)	LH (mm)	+/- (%)	TI (%)
<i>Brassica oleracea</i>	Control	31,3±1,62	0.00	23,15±1,44	0,00	-
	V1(60mg/l)	12,8±0,87*	- 59,11	18,6±0,89	-19,66	40,89
	V2 (100mg/l)	9,4±0,74*	- 69,97	15,25±0,91*	-34,13	30,03
	V3 (150mg/l)	7,8±0,81*	- 75,08	14,4±0,93*	- 37,8	24,92
<i>Cucurbita pepo</i>	Control	48,1±3,31	0,00	64,1±4,15	0,00	-
	V1(60mg/l)	34,05±2,58*	- 29,21	45,2±3,19*	- 29,49	70,79
	V2 (100mg/l)	21,75±2,57*	- 54,79	33±2,46*	- 48,52	45,21
	V3 (150mg/l)	16,1±2,15*	- 66,53	26,75±2,25*	- 58,27	33,47

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

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, cultivar Roma VF; Gang *et al.*, (2013) at the wheat root, variety GW-366. According to Maksimiec (1997), the copper ions in excess

inhibit to a large extent the elongation, inducing the formation of free radicals that cause oxidative stress and affect the permeability of the membranes. The stress caused by heavy metals reduces the vitality of the root and affects the process of growth (Cheng, 2003).

The tolerance index (TI) presented in both species a gradual decrease in value, with the increase of the metal concentration. The pumpkin, comparing with the white cabbage, presented a higher tolerance in the concentrations of copper used (Table 2). A low tolerance to high concentrations of copper was reported at: chickpea and *Phaseolus mungo* (Kumar *et al.*, 2009); tomatoes (Asharge *et al.*, 2013). According to Cheng (2003), plants have their own mechanisms of detoxification of the heavy metals; these mechanisms are integrated and protect the plants against the negative effects caused by heavy metals.

The chlorophyll fluorescence. At the concentration of 150 mg/l were recorded the highest values of the parameters F_s and F_m' , fact that suggest an unfavourable influence of the copper on the functioning of the photosynthetic apparatus. It was noticed a decrease in value of the parameter $\Phi PSII$, insignificant at concentrations of 60 mg/l and 100 mg/l and significant at the concentration of 150 mg/l (Table 3). This fact indicates a sensitivity of the activity of the photo system II in the chloroplasts of the cotyledon leaves at the treatment applied. It is considered that the parameter $\Phi PSII$ is very sensitive to the excess of heavy metals (Vasiliev and Manolov, 1999).

Table 3

The fluorescence parametres at pumpkin

Species	The variant	The analised indicator		
		F_s (bits)	F_m' (bits)	$\Phi PSII$
<i>Cucurbita pepo</i>	Control	636,66 (100)	3416 (100)	0,813 (100)
	V1(60mg/l)	638 (100,21)	3222,33 (94,33)	0,801 (98,52)
	V2 (100mg/l)	586 (92,04)	3075,66 (90,03)	0,809 (99,50)
	V3 (150mg/l)	785,66 (123,40)	3694,33 (108,14)	0,787* (96,80)

Note:* indicates significant differences (Tukey test, $p < 0.05$); the number in brackets represents the percentage relative to control

CONCLUSIONS

1. The copper in the concentrations used did not affect the germination, but it influenced unfavourably the growth of seedlings and the parameters of the chlorophyll fluorescence.

2. The two test species presented a similar evolution of the sensitivity comparing with the increase in the metal concentration. *Cucurbita pepo* comparing with *Brassica oleracea* was less sensitive at the applied concentrations.

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