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RESEARCH ON THE INFLUENCE OF $CuCl_2$ ON THE SEED GERMINATION OF *DIANTHUS SUPERBUS* L. AND *GLOBULARIA PUNCTATA* LAPEYR

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Abstract

The aim of the researches was to establish the upper limit of $CuCl_2$ concentration in soils that the Dianthus superbus L. and Globularia punctata Lapeyr. species could tolerate without significantly affecting the germination and the plant sprouting process. The experiment was conducted in 4 variants of 3 repetitions, each repetition including 50 seeds. In order to moisten the substrate, water was used for the control variant and for the other three variants, solutions of different CuCl2 concentrations were used. The necessary quantity of $CuCl_2$ was calculated, in order to obtain certain concentrations in the substrate V_2 - 0.1 mM; V_3 - 1 mM and V_4 – 10 mM. Testing seed germination under $CuCl_2$-polluted conditions has led to the study of all species studied to obtain a percentage of germination that decreased with increasing concentration. The increase in $CuCl_2$ concentrations in the germination substrate in Dianthus superbus induced a slight delay in the emergence and a shift in the number of days in which the rising occurred.

Key words: *Dianthus superbus, Globularia punctata, germination, velocity.*

INTRODUCTION

Water and soil pollution is a major global problem and is the leading cause of disease causing death worldwide (Pink Daniel, 2006). Certainly, soil and environmental pollution has increased with the demographic explosion and, if no action is taken, things will get out of hand.

The pollution of ecological systems with heavy metals is a major problem due to its influence on the functioning of biocenosis, as well as the penetration of heavy metals into the structure of food chains (Adams et collab., 2000). Numerous studies have reported the germination test as a basic method for determining the effects of Cu toxicity on many plant species.

Reducing the percentage of germination as a result of the presence of copper in the germination environment has been highlighted in different species: *Triticum aestivum* L. (Gang et collab., 2013; Singh et collab., 2007); *Vicia sativa* L. (Muccifora and Bellani 2013); *Vigna radiata* L. (Verma et collab., 2011); *Phaseolus vulgaris* L. (Ashagre et collab., 2013); *Oryza sativa* L. (Ahsan et collab., 2007; Mahmood et collab., 2007); *Glycine max* L and *Cicer arietinum* L. (Adhikari et collab., 2012).

The results of specialized studies suggest that the toxicity of Cu in the seed germination process in different plants may exhibit a remarkable variability of tolerance both within the genus and between different species (Ansari et collab., 2013). In the case of *Vicia sativa* L., germination decreased as the concentration of Cu increased to 5 × 10-3 M (Muccifori and Bellani 2013).

In the case of *Vigna radiata* L. it was noted that the percentage of seed germination decreased with the increase of Cu concentrations (50, 200 and 500 μM in the nutrient solution at a 72 hours exposure time (Verma et collab., 2011). Similar results were obtained in the germination of *Oryza sativa* L. seeds, studies having shown a decrease in the germination percentage with an increase in the concentration of Cu from 0.2 mM to 1.5 mM (Ahsan et collab., 2007).

A similar tendency of decrease in the germination of seeds was observed in some wheat and rice varieties in the case of which the germination percentage was reduced to 60%

and 35% when the substrate was contaminated with a copper concentration of 10 µM (Mahmood et collab., 2007).

From the results of the studies carried out on soybean seeds (*Glycine max* L.) and chickpea (*Cicer arietinum* L.) it can be concluded that the excess of Cu induces a reduction of the germination capacity (Adhikari et collab., 2012).

MATERIALS AND METHODS

For the experiment, the biological material used was represented by seeds of *Globularia punctata* and *Dianthus superbus* L., species preserved ex-situ in the collection of the Floriculture department of the Faculty of Horticulture.

The experiment used containers of 2 kg capacity, containing soil, being structured in 4 variants of 3 replicates each, each repetition having 50 seeds.

For the watering of the substrate, water was used in the control variant and solutions of different concentrations of $CuCl_2$ were used in the other three variants. To test the influence of $CuCl_2$ on seed germination, the following concentrations were used: 0.1 mM (V_2), 1 mM (V_3), 10 mM (V_4).

Seed germination was done in the SANYO germinator (MLR-351H) at a temperature of 22 ± 1 ° C for 8 hours, 24 ± 1 ° C for 16 hours, a relative air humidity of about 80% and a luminous intensity of over 8,000 lux.

During the experiment, daily determinations were made regarding the germination percentage, germination rate, velocity and velocity coefficient (Kotowski, 1962).

RESULTS AND DISCUSSIONS

In the case of *Dianthus superbus*, the results regarding the germination percentage indicate very low values, for the uncontaminated variant included, indicating that germination is influenced by the climatic conditions of the maturation period of the seeds used in the experiment.

The testing of this species' germination was also studied by Zaharia A., 2014, who obtained a much better germination percentage, 81%, using seeds from the natural habitat from where the species was taken for preservation.

Under conditions of contamination, for the *Dianthus superbus* species, a decrease in the germination percentage was obtained, depending on the increase in the concentration of $CuCl_2$. From the determinations carried out, the best germination percentage was found in the variant where the substrate was watered with 0.1mM (40%) $CuCl_2$ concentration, and the lowest germination percentage was found in the variant with the highest $CuCl_2$ concentration (V_4 by 21%).

Compared to the control variant, experimental variant V_2 registered an increase in the germination percentage of 6% suggesting that the presence of a low concentration of $CuCl_2$ in the substrate stimulates the plant germination (table 1).

In the other experimental variants, either the same percentage of germination as the one of the control variant (V_1 by 35%) or a much lower germination than the one of the control variant (V_4 by 21%) was obtained.

The triggering of the sprouting started faster in V_2 (6 days after sowing) and it was delayed in V_4, reaching up to 8 days (table 1).

Table 1. Influence of copper on the seed germination of the *Dianthus superbus* species

Variant	Sowing date	Germination onset date	End of germination date	Total germination *
V_1	8.04.2015	15.04.2015	21.04. 2015	35
V_2	8.04.2015	14.04.2015	21.04. 2015	40
V_3	8.04.2015	15.04.2015	23.04. 2015	33
V_4	8.04.2015	16.04.2015	23.04. 2015	21

*The values represent % of normal sprouts

The sprouting period was 7 days in the variant for which the substrate was watered with water (control variant), 6 days in variant V_2 and 8 days in variant V_3 and V_4 (figure1).

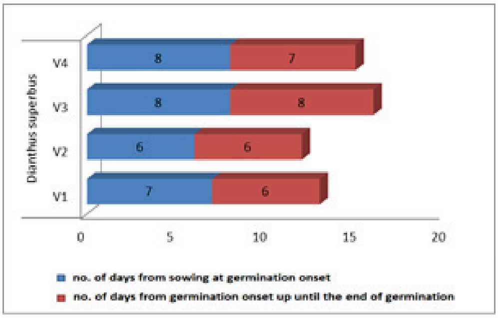

Figure 1. Seed germination duration under conditions of copper contamination (number of days)

The influence of $CuCl_2$ on seed germination in the case of *Dianthus superbus* was also highlighted by the analysis of the following indicators: the sprouting dynamics, sprouting rate and velocity of seedling. The results regarding the sprouting dynamics for this species are presented in table 2.

Table. 2. The dynamic emergence of *Dianthus superbus*

Variant	Sowing date	Date/sprouted plants (%)									
		14.04	15.04	16.04	17.04	18.04	19.04	20.04	21.04	22.04	23.04
V_1 (control)	08.04	-	4	7	11	16	27	32	35	35	35
V_2	08.04	2	5	9	16	20	29	36	40	40	40
V_3	08.04	-	4	7	13	17	26	29	31	32	33
V_4	08.04	-	-	3	6	8	12	17	20	20	21

The onset of sprouting occurred 6 days after sowing (14.04) in variant V_2, one day later for the control variant and V_3 and two days later for V_4 (16.04). In variants V_1, V_2 and V_3, 10 days after sowing (18.04), the number of sprouted plants exceeded 50% of the final percentage. For V_4, it is noted that the percentage of sprouted plants emerging from day to day is very low and decreases as it approaches the end of the sprouting period.
From the determinations regarding the sprouting rate, a maximum percentage of 11% was noted in the control variant, 11 days after sowing and at the end of the sprouting period, it was 2%.
For variants V_2 and V_3, the maximum percentage of the sprouting rate was 9%, also 11 days after sowing.
Concerning variant V_4, the percentage of the sprouting rate varied from 1% at the end of the sprouting period, to 5%, 12 days after sowing (table 3).

Table 3. The sprouting rate of the *Dianthus superbus* species under exposure to different doses of $CuCl_2$

Variant	Sowing date	Date/ sprouting rate (%)									
		14.04	15.04	16.04	17.04	18.04	19.04	20.04	21.04	22.04	23.04
V_1 (control)	08.04	-	4	3	4	5	11	5	3	0	0
V_2	08.04	2	3	4	7	4	9	7	4	0	0
V_3	08.04	-	4	3	6	4	9	3	2	1	1
V_4	08.04	-	-	3	3	2	4	5	3	0	1

By comparing the data obtained from the four experimental variants in the calculation of the dynamics of the sprouting velocity (table 4), it is noted that in V_2 the velocity values reached 3,07% and 2,66% in the control variant.
In the variants for which the seeds germinated in the contaminated substrate with the highest concentrations of $CuCl_2$, the velocity values were up to 2.42% for V_2 and 1.54% for V_4.

Table 4. General characterization of the seedling of the *Dianthus superbus* species under exposure to different doses of $CuCl_2$

Variant		Date									
		14.04	15.04	16.04	17.04	18.04	19.04	20.04	21.04	22.04	23.04
V_1 (control)	V	-	0,57	0,87	1,22	1,6	2,45	2,66	2,69	0	0
	C_v	-	1,68	2,51	3,59	4,7	7,21	7,84	7,69	0	0
V_2	V	0,33	0,71	1,12	1,77	2	2,63	3	3,07	0	3,07
	C_v	0,83	1,78	2,81	4,44	5	6,59	7,5	7,69	0	7,69
V_3	V	-	0,57	0,87	1,44	1,7	2,36	2,42	2,38	2,28	2,20
	C_v	-	1,68	2,51	4,24	5	6,95	7,11	7,01	6,72	6,66
V_4	V	-	-	0,37	0,66	0,8	1,09	1,42	1,54	0	1,40
	C_v	-	-	1,70	3,03	3,63	4,95	6,44	6,99	0	6,66

V - velocity

Cv – coefficient of sprouting velocity

Regarding the coefficient of sprouting velocity, a trend similar to velocity was observed, from one variant to the other, as well as at the level of similar variants.

The highest values of the coefficient of velocity were recorded for variants V_1 (7.84%), and V_2 (7.69%) and the lowest values were recorded for variant V_4 with 6.99%.

The results of the research regarding the *Globularia punctata* seed germination in a substrate contaminated with different concentrations of $CuCl_2$ highlight the adaptation of this species to the toxicity of this pollutant.

In the case of *Globularia punctata*, the studies regarding the seed germination percentage from the spontaneous flora show a very good germination rate of 75% (Zaharia A., 2014). Regarding the results of the determinations carried out within the experiment, a low germination percentage is observed in all variants. The results suggest that the percentage of germination depends not only on the conditions under which the experiment is carried out, but also on the environmental conditions under which the seeds had matured.

The sprouting began 8 days after sowing (16.04) in the case of V_2, 9 days after sowing (17.04) for V_4 and 10 days after sowing for the control variant and V_3 (18.04).

The end of the sprouting period varied from 14 days for the control variant and V_2 to 19 days for variant V_4 (table 5).

Table 5. Influence of copper on the seed germination of the *Globularia punctata* species

Variant	Sowing date	Germination onset date	End of germination date	Total germination *
V_1	8.04.2015	18.04.2015	23.04. 2015	30
V_2	8.04.2015	16.04.2015	22.04. 2015	26
V_3	8.04.2015	18.04.2015	25.04. 2015	21
V_4	8.04.2015	17.04.2015	27.04. 2015	20

*The values represent % of normal sprouts

Regarding the germination percentage, it varied from 20% for variant V_4 to 30% for the uncontaminated variant.

The highest germination percentage was registered in the plants from the uncontaminated variant (30%).From the analysis of the results obtained it is noted that the presence of $CuCl_2$ determines a general tendency of decrease in the germination percentage.

The variants treated with $CuCl_2$ showed a 4% decrease in the V_2 variant, a 9% decrease in the V_3 variant and a 10% decrease in the V_4 variant, compared to the control variant.

In the case of variants V_2 and V_4 which had a 26% sprouting rate compared to the control variant, respectively 21% for V_3, the sprouting was triggered for both variants 10 days after sowing (18.04) and went on for 14 days for V_2 (22.04) and for two days more, for V_4 (25.04). For V_3, the sprouting started 8 days after sowing and happened over 15 days.

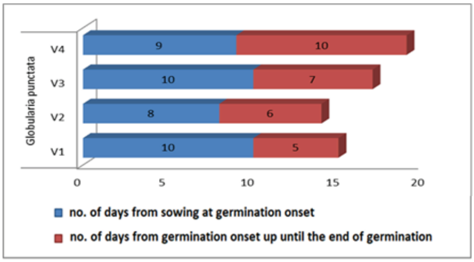

Figure 2. Seed germination duration under conditions of copper contamination (number of days)

The longest sprouting period was highlighted in the case of variant V_4 for which the higher $CuCl_2$ dose induced the delay of the ending of the sprouting period with up to 19 days (figure 2).

For the control variant the maximum percentage of sprouted plants from day to day was recorded in the 13-14 days after sowing, and at the end of the sprouting period it was 30%.

For V_2 the maximum percentage of sprouted plants from day to day was recorded starting from day 10 and was maintained until the end of the sprouting period, when a 26% of germinated seeds was obtained.

For variants V_3 and V_4, the maximum percentage of sprouted plants was recorded on days 5 and 6 after sprouting and it ended with a percentage of 21% for V_3 and 20% for V_4 (table 6).

Table. 6. The dynamic emergence of *Globularia punctata*

Variant	Sowing date	Date/sprouted plants (%)											
		16.04	17.04	18.04	19.04	20.04	21.04	22.04	23.04	24.04	25.04	26.04	27.04
V_1 (control)	08.04	-	-	2	4	9	15	23	30	30	30	30	30
V_2	08.04	1	3	7	11	15	19	26	26	26	26	26	26
V_3	08.04	-	-	2	5	10	14	18	20	20	21	21	21
V_4	08.04	-	4	5	8	11	15	17	18	19	19	19	20

Regarding the sprouting rate, the percentage varied in the case of the control variant from 2%, at the beginning of the sprouting period, to 5% at 11 and 12 days after sowing.

In the case of V_2, the percentage of the sprouting rate was 1% at the beginning of the sprouting period, starting on the third day of sprouting, got to 4% and then increased to 57% on the last day of sprouting.

For variant V_4, the percentage of the sprouting rate varied from 1% at the end of the sprouting period to 4% at 13 days after sowing (table 7).

Table 7. The sprouting rate of the *Globularia punctata* species under exposure to different doses of $CuCl_2$

Variant	Sowing date	Date/sprouting rate											
		16.04	17.04	18.04	19.04	20.04	21.04	22.04	23.04	24.04	25.04	26.04	27.04
V_1	08.04	-	-	2	2	5	6	8	7	0	0	0	0
V_2	08.04	1	2	4	4	4	4	7	0	0	0	0	0
V_3	08.04	-	-	2	3	5	4	4	2	0	1	0	0
V_4	08.04	-	4	1	3	3	4	2	1	0	1	0	1

From the analysis of the results regarding the dynamics of the velocity of sprouting (table 8), we note that in the case of the control variant, V_2 and V_3, the values of the velocity reached 1.33% and for variant V_1, we note values reaching 1.71%.

As for *Dianthus*, for this species also, the coefficient of sprouting velocity showed a tendency similar to the one of velocity, both in the case of the control variant and the contaminated variants.

The highest values for the coefficient of velocity were recorded in variants V_3, with 7.00%. In the case of the other two variants (V_1, V_2) the maximum percentage of the coefficient of velocity was 6.66% (table 8).

Table 8. General characterization of the seedling of the *Globularia punctata* species under exposure to different doses of copper

Variant		Date %											
		16.04	17.04	18.04	19.04	20.04	21.04	22.04	23.04	24.04	25.04	26.04	27.04
V_1 (control)	V	-	-	0,2	0,36	0,75	0,46	0,57	0,46	0	0	0	0
	C_v	-	-	1	1,81	3,75	3,85	5,47	6,66	0	0	0	0
V_2	V	0,12	0,33	0,7	1	1,25	1,46	0,5	1,6	0	0	0	0
	C_v	0,52	1,38	2,91	4,16	5,20	6,08	7,14	6,66	0	0	0	0
V_3	V	-	-	0,2	0,45	0,83	1,07	1,28	1,33	1,25	1,24	0	0
	C_v	-	-	6,95	2,16	3,96	5,12	6,12	7,00	6,56	5,88	0	0
V_4	V	-	0,44	0,5	0,72	0,25	0,31	0,14	0,06	0	0,58	0	0,53
	C_v	-	1,93	2,17	3,16	4,58	5,77	6,07	6,00	0	5,94	0	5,26

V – velocity Cv – coefficient of sprouting velocity

CONCLUSIONS

Testing seed germination under conditions of $CuCl_2$ pollution has determined in all studied species a percentage of germination that has decreased with the increase of $CuCl_2$ concentrations.

The increase in $CuCl_2$ concentrations in the germination substrate for *Dianthus superbus* induced a slight delay in sprouting and a shift in the number of days in which the sprouting occurred.

In the case of *Globularia punctata*, higher $CuCl_2$ doses resulted in a larger shift in the number of days in which the sprouting occurred, of 5 days in V_4 and 2 days in V_3.

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