RESEARCH ON THE EFFECT OF COPPER ON GERMINATION AND GROWTH OF *Phaseolus vulgaris* var. Nanus

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Abstract.

Copper has been and continues to be widely used in agriculture due to its antifungal and antibacterial properties. In the same time, foliar fertilizers, many of which contain a significant amount of copper, are applied to crops during the vegetative stage in order to increase efficiency. Therefore, providing copper to crop plants prevents the appearance of diseases and increases crop production; however, because copper is a heavy metal, it accumulates in the soil over time, reaching toxic concentrations, resulting in increased mobility in soil solution and increased bioavailability for plants. The effects of toxic copper levels on plants are not fully understood, but they have sparked serious concern among scientists. The goal of the study was to see how different copper concentrations affected the germination and growth of seedlings of Phaseolus vulgaris var. Nanus in their early stages of development.

Key words: abiotic stress, copper, germination, heavy metal, *Phaseolus*

INTRODUCTION

Copper is a heavy metal that is widely used in agriculture as a fungicide for the treatment of seeds and plants, as a foliar fertilizer, and as an algaecide in water treatments. Copper has been and continues to be used in agriculture as a result of its antifungal and antibacterial properties, as well as its high efficiency and low cost, in the form of various commercial products based on: hydroxide Cu, oxychloride, or Cu sulphate. However, copper accumulates in the soil over time, reaching toxic concentrations (Ballabio et al., 2018), resulting in increased mobility of the soil solution and increased bioavailability to plants. According to the ICPA Soil Monitoring Report, the average copper content in 670 agricultural land sites in the country is 26.07mg/kg dry soil, in conditions where, according to MAPPM Order no. 756/1997, the normal value of the Cu concentration in the soil in Romania is 20 mg/kg dry soil, the values considered alert thresholds are 100 - 200 mg/kg d.s. and the values for intervention are 250 – 500 mg/kg d.s. Thus, 48.7% of the analyzed sites had normal copper contents, 50.6 % were between the normal value and the alert threshold for sensitive use, and 0.44 %(i.e. 3 sites) had values above the alert threshold, while only one site exceeded the threshold intervention, recording 551mg Cu/kg soil (Dumitru et al., 2011).

Under normal conditions, Cu is found in plant tissues at concentrations of 10 ug/g s.u. (Baker and Brooks, 1989, Kanoun-Boulet et al., 2009), being an essential element with an important role in the synthesis of chlorophyll and enzymes, as well as in photosynthesis, respiration, and the metabolism of carbohydrates, proteins, and cell wall constituents, or in lignification (Yruela, 2005). As a result, copper deficiency can alter various metabolic functions in plants (Rehman et al., 2019). Light spots were also observed on the leaves, and in the case of straw cereals, white and empty ears, as well as delaying twinning and reducing resistance to falling (Scăeţeanu and Pele, 2013). However, high copper concentrations in soil are toxic to most plants and slow plant growth due to changes in mineral nutrition, photosynthesis, enzymatic activities, and decreased chlorophyll synthesis (Adrees et al., 2015). Its toxicity stems primarily from the possibility of forming free radicals, which cause oxidative stress in plants (Fernandez and Henriques, 1991). Shen et al. (1998) demonstrated that excess Cu can have a variety of direct and indirect effects on the metabolism on *Vigna radiata* plants. Aladessanni et al. (2019) discovered an increase in Cu content of plants while studying the phenomenon of heavy metal bioaccumulation in corn (*Zea mays*), which affects not only productivity but also the quality and safety of food. As a result, the continuous release into the environment and on a large scale of agrochemicals based on copper, which have traditionally and sometimes excessively been used in agriculture, has become an urgent problem, with numerous cases of phytotoxicity documented. Previous research by Xu et al. (2006), Verma et al. (2011), Thounaojam et al. (2012), Bharwana (2015), and others has shown that increased copper levels in soil can affect plant growth, significantly reduce crop productivity, and even threaten human health by penetrating the food chain.

MATERIAL AND METHOD

The plant material used in the current experiments was seeds of *Phaseolus vulgaris* L. var. Nanus (*Fabaceae)*, a plant native to South America that is now grown all over the world (Graham and Ranolli, 1997).

To conduct the experiment, we used transparent plastic pans with lids measuring 22/14 cm and h = 6 cm. In each casserole, we used absorbent paper as a base and evenly distributed 50 grains. The experimental group also included a control variant (V_0), which was only watered with distilled water throughout the experiment. According to the experimental protocol shown in Table 1, we used different concentrations of active substance (0.1%, 1%), applied foliar or root, for the other three variants.

The chemical substance that we used was fungicide CHAMP 77 WG (50% metallic Cu from copper hydroxide) dissolved in distilled water for the experiment.

Table 1

Experimental protocol (DW– distilled water; Cu –copper, active substance; T– temperature).

Germination conditions	**Experimental variants and mode of application**	**Biometrics**	**Time for measurements**
T = 22° ± 2°C Natural light	*V_0 - radicular – **DW** on the first day* *- foliar – **DW** on the 7^{th} day* V_1**– radicular – 0.1% Cu** (100mg/l) on the first day - foliar – DW on the 7^{th} day V_2 - **radicular - 1 % Cu** (1000 mg/l) on the first day - foliar – DW on the 7^{th} day V_3 – radicular – DW on the first day **- foliar – 0.1% Cu** (100 mg/l) on the 7^{th} day	**Germination rate**	24h 48 h 72 h 5 days 7 days 10 days 14 days
		Growth indices: - embryo root length - adventitious roots number - hypocotyl length - epicotyl length - leaves number - plant size - dry weight	14 days

The abiotic stress to which the beans from variants V_1 and V_2 were subjected consisted of watering them at the base by how many 20 ml of liquid (3 ml of solution + 17 ml of DW) from the concentration solution corresponding to each experimental variant on the first day of the experiment. On all other days, we used only distilled water.

For V_3 variant we used the same concentration as in variant V_1 (3 ml of 0.1% solution), applied foliar on the seventh day of the experiment. For watering on the rest days, we used only distilled water, in the same amount as in the case of variants V_1, V_2, and V_0 (control). On the same day of the experiment (7^{th}), we used the same amount (3 ml) of distilled water for foliar application of seedlings for all other experimental variants (V_0, V_1, V_2).

Following the completion of the experiment and removal of the remaining material seedlings, the seedlings of each experimental batch were wrapped in aluminum foil and kept in the oven at 115°C, for 4 hours, for 3 consecutive days, after which the dry weight was determined by weighing on an analytical balance.

Data from biometric measurements were mathematically and statistically processed in the Microsoft Excel 2010 program, with the average arithmetic, difference from control, standard deviation, and student test calculated (*t* test).

RESULTS AND DISCUSSION

The germination process in all experimental variants started 24 hours after the seeds were planted and ended 10 days after the experiment began (Fig. 1). The rate of germination was found to be greater than 96% for all experimental variants at the end of the experiment, indicating that Cu treatment of applied copper hydroxide root or leaf did not inhibit the germination process in beans (Fig. 1).

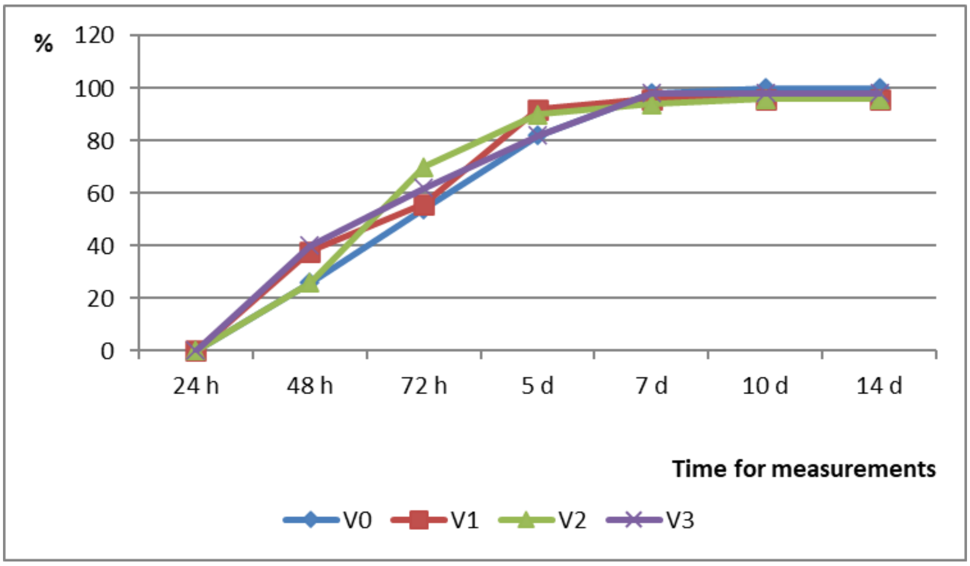

Fig.1. Monitoring the germination rate of bean (*Phaseolus vulgaris* var. Nanus) seeds, on the following experimental variants: V_0 – distilled water; V_1 – 0.1% Cu, radicular application; V_2 - 1% Cu, radicular application; V_3 – 0.1% Cu, foliar application, at different time intervals (h-hours, d-days).

Verma et al. (2011) found similar results when they followed the germination of mung bean seeds (*Vigna radiata*) treated with different concentrations of Cu in copper sulfate and discovered that the percentage of germination was over 95%, indicating that the germination process was not inhibited by different concentrations of copper. Similar findings were reported by Saquoir et al. (2008), who investigated the toxic potential of copper on *Vicia faba* and *Pisum sativum* plants in hydroponic culture conditions.

In terms of the growth and development indices of seedling beans, the root-treated batch with 1% Cu showed very statistically significant negative differences compared to the control group, both in terms of the length of the embryo root, the length of the hypocotyl, and, implicitly, seedling size and number of leaves (Table 2).

Table 2

Statistical processing of bean (*Phaseolus vulgaris* var. Nanus) growth index values, 14 days after seeding to germinate on different experimental variants: V_0 – distilled water; V_1 – 0.1% Cu, radicular application; V_2 - 1% Cu, radicular application; V_3 – 0.1% Cu, foliar application.

Variants	Average ± standard deviation						Difference from control (cm)/ significance of difference					
	Embryo root length *(cm)*	**Adventi-tious roots number**	**Hypocotyl length *(cm)***	**Epicotyl length *(cm)***	**Leaves number**	**Plant size *(cm)***	**Embryo root length *(cm)***	**Adventi-tious roots number**	**Hypocotyl length *(cm)***	**Epicotyl length *(cm)***	**Leaves number**	**Plant size *(cm)***
V_0	5.41 ± 4.33	3.92 ± 2.01	1.98± 2.11	0.05 ± 0.13	0.48 ± 0.86	7.44 ± 5.89	-	-	-	-	-	-
V_1	4.14 ± 3.47	4.14 ± 1.75	1.89± 2.14	0.06 ± 0.19	0.56 ± 0.91	6.09 ± 5.13	-1.27 **	0.22 *	-0.09 **	0.01 *	0.08 *	-1.35 *
V_2	1.50 ± 0.80	3.68 ± 1.56	0.87± 0.31	0.00 ± 0.00	0.00 ± 0.00	2.38 ± 1.01	**-3.9 *** **	-0.24 *	**-1.11 *** **	-0.05 **	-0.48 ***	**-5.06 *** **
V_3	4.47 ± 3.58	3.92 ± 1.74	2.57± 2.72	0.23 ± 0.52	0.87 ± 1.00	7.26 ± 6.19	-0.94 *	0.00 ns	**0.59** *	**0.18 ****	**0.36 ****	-0.17 *

Note: *** p<0.01 - very significant; ** p<0.1 - distinctly significant; * p<0.5 – significant; ns p>0.5 – no statistically significant difference.

At 7 days, both the seedlings in the group treated with root with 0.1% Cu and those in the foliar treated group with the same concentration of metal not only did not show inhibitions, but even recorded a significant increase over the group control, demonstrating that the toxic effect of copper on plants beans is manifested only at high concentrations, less in the case of length embryo root.

Because the values in the tables were only mentioned with two decimals in mathematical processing, statistical results appear even at apparent values of 0.00 (Table 2).

In the case of testing the manufacturer's recommended concentration, but with root application, encoded V_1 in our case, the rhizogenesis was inhibited, with average deficits of 1.27 cm recorded compared to the control, these being statistically significant (Table 2). High concentration of Cu, similar to the alert threshold tested in group V_2, conducted to high deficits, both in terms of rhizogenesis and caulogenesis, according to statistical data (Table 2), with these inhibitions also found in seedling dry weight values (Table 3).

These findings are consistent with previous research, but we also observed growth increases in seedlings treated with the recommended concentration of Cu, indicating that Cu acts as a micronutrient with stimulating effects on development bean seedlings. Iqbal et al. (2018) observed inhibitory effects on lentil root growth (*Lens culinaris*) at concentrations of 25 mg Cu/l, but only at high Cu concentrations (75 mg/l and 100 mg/l) were significant toxic effects on stem growth observed. In addition, Oros et al. (2011), in a study on *Medicago sativa* plants, concluded that at low concentrations, copper has a stronger toxic effect on the stem and a weaker inhibitory effect on the root, and at high concentrations, the relationship is reversed, with the inhibitory effect being stronger on the root.

The dry weight data (Table 3) support the inhibitory effect of increased copper concentration on the growth of bean seedlings.

Table 3

Dry weight of bean (*Phaseolus vulgaris* var. Nanus) seedlings, 14 days after seeding to germinate on different experimental variants: V_0 – distilled water; V_1 – 0.1% Cu, radicular application; V_2 - 1% Cu, radicular application; V_3 – 0.1% Cu, foliar application.

Experimental variants	**Dry weight (g)**
V_0	1.9887
V_1	1.8930
V_2	**0.9295**
V_3	**2.0077**

Thus, the batch treated with 1% Cu root had the lowest value compared to the control group (Table 3), as well as the batch with the strongest inhibitions of growth and development indices (Table 2). This decrease in yield is associated with an increase in copper in soil, indicating that copper has a high level of phytotoxicity to plants at high concentrations. Other researchers have reported a significant reduction in plant biomass under high copper conditions (Baszynski et al., 1988; Lidon and Henriques, 1991).

At the same time, the batch treated with 0.1 % Cu, the manufacturer's recommended concentration, had a value similar to that of the control batch, and in cases of foliar-treated seedlings, after 7 days, with the same concentration of copper recorded an increase compared to the value of the control group (Table 3).

CONCLUSIONS

1. The presence of copper, regardless of the concentrations tested, influenced lightly the bean germination rate.
2. Copper generated increases and inhibitions of growth rates in the dicotyledonous species in low concentrations (0.1%), radicular applied, but the overall size of the seedlings and their dry weight were found to be inferior to the control group, at the end of the experiment.
3. The toxic effect of copper was found at the high concentration of copper (1%), radicular applied on the first day, causing significant inhibitions of the growth indices, both from the perspective of rhizogenesis and caulogenesis.
4. Foliar application of 0.1% Cu solution, according to agricultural crop recommendations, on the seventh day of the experiment, after the appearance of the first leaves, was a significant incentive for bean seedlings, only in terms of caulogenesis and dry weight.

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