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OBSERVATIONS CONCERNING THE EFFECTS OF THE FOLIAR APPLICATION OF COPPER ON SOME PHYSIOLOGICAL PROCESSES OF *CALENDULA OFFICINALIS* L. SPECIES

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***Key words**: Calendula officinalis L., aqueous solution of copper sulfate, assimilating pigments, dry matter*

INTRODUCTION

Micro-elements are used in agriculture depending on the physical and chemical characteristics of the soil, on the anatomo - physiological peculiarities of plants and on the assimilable forms of micro-elements. Micro-elements are applied as chemical compounds resulting from the extraction of a specific substance. They are fed to the plants as aqueous solutions, either by treating the soil/seeds before sowing or by spraying the plants (Pop E., Peterfi Șt., 1964).

In literature, the specialists point at the multiple roles of micro-elements in plant life: they participate in the formation of enzymes, vitamins and hormones, increase the resistance of plants to diseases, influence the photosynthesis process, stimulate sap circulation, growth and flowering (Seciu T., Hemegariu O., 1967). Applying micro-elements in agriculture is currently protected and there are several researches explaining their mechanism of action, optimal dosage and application techniques for different species (Milică C.I. et al., 1982). The usage of micro-elements on medicinal plants growing has been recently investigated. This is why we intend to test the effects of foliarly applied copper on some physiological processes for *Calendula officinalis*.

Calendula officinalis L. is a species originated in Mediterranean and west-Asian regions. It is widespread over most European regions as an ornamental plant, but it can be met as a subspontaneous species (Pârvu C., 2003). Calendula is cultivated as a medicinal herb in Germany, Czech Republic, Slovak Republic, Poland, Bulgaria, Hungary, Austria, Syria and Egypt. The plant has a tall richly branched, pubescent stem of 40–60 cm, with strong balsamic perfume (Mencinicopschi Gh., Bojor O., Ionescu Larisa, 2009).

MATERIAL AND METHODS

Our plant physiology research was carried out on plants of *Calendula officinalis*.

These plants were obtained by seed germination in plastic pots with soil purchased from flower shops.

Calendula officinalis seeds were purchased from stores that sell seed material and their authenticity is guaranteed by the producing company.

The physiological processes of plant germination and growth developed under controlled conditions in a SANYO MRL351H growth chamber, where they were submitted to a 20^{o}C temperature, a photoperiod of 4 hours of light and 8 hours of darkness, at a light intensity of 22 lux. After their plantation in plastic pots with potting soil, the seeds were watered twice a day with tap water, until the seedlings and two true leaves emerged. When seedlings developed, they were removed from the growth chamber and treated with an aqueous copper sulfate solution in different concentrations as indicated in Table 1.

The plants were treated with $CuSO_4$ aqueous solution in different concentrations during four successive days, in the mornings, using variants to observe the effects of copper as salt ($CuSO_4$) on some physiological processes, but the control sample was watered with tap water. For a single spraying and watering, 2 ml solution of copper sulfate was used. At the end of the application of the copper sulfate solution, the content of dry matter and assimilating pigments in the green parts of the plant were established. 20 days after the experiment started, the treated plants were biometrically measured, with particular interest in the length of root, hypocotyl, stem and cotyledons.

The length was measured in centimetres with a ruler, and the data were processed mathematically in Microsoft Excel.

For each biometric index, the arithmetic mean (x) was calculated as the mean value for the parameter analyzed, resulting from the sum of individual values divided by the total number of individuals.

Table 1. Treatment of *Calendula officinalis* plants with aqueous solutions of micro-elements

Treatment \ Variant		V_0	V_1	V_2	V_3
Tap water	Watering	2 ml	-	2 ml	-
	Spraying	1 x	1 x	-	-
0.10% $CuSO_4$ aqueous solution	Watering	-	2 ml	-	2 ml
	Spraying	-	-	1 x	1 x
0.15% $CuSO_4$ aqueous solution	Watering	-	2 ml	-	2 ml
	Spraying	-	-	1 x	1 x
0.25% $CuSO_4$ aqueous solution	Watering	-	2 ml	-	2 ml
	Spraying	-	-	1 x	1 x

1 x = once

The quantitative determination of assimilating pigments from *Calendula officinalis* L. was done by spectrophotometric Mayer-Bertenrath method adapted by Ştirban and Fărcuş, using 85% acetone in the extraction of pigments by preparing the vegetal material with $CaCO_3$ to prevent the transformation of chlorophylls in pheophytines.
The dry matter and water contents were established by the gravimetric method, stove drying at 105ºC.

RESULTS AND DISCUSSIONS

The dry matter content in the green parts of the plants registered values ranging between 4.97 g% dry matter (sample V1 - 0.10% $CuSO_4$ aqueous solution) and 23.54 g% dry matter (sample V3 – 0.25% $CuSO_4$ aqueous solution) (Fig. 1).

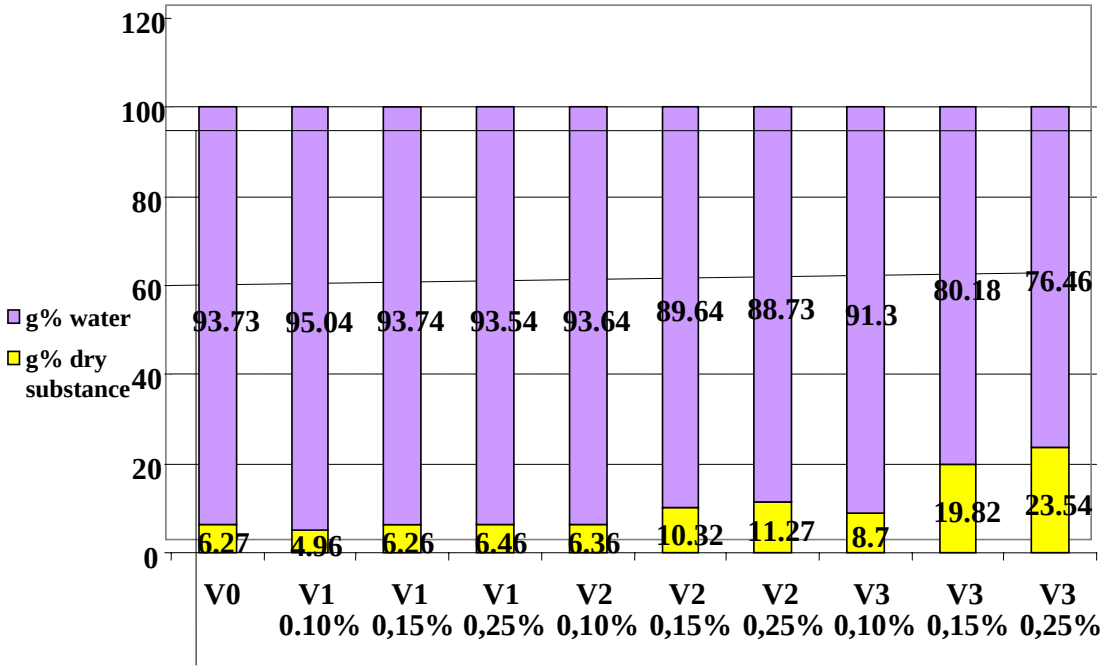

Fig. 1. Dry matter and water content *Calendula officinalis* L. plants treated with $CuSO_4$ solution in different concentrations

The dry matter parameter registered similar values of over 6 g% dry matter on the following tested variants:

- V0 (control sample) – 6.27 g% dry matter;
- V1 (0.15% $CuSO_4$ solution) – 6.26 g% dry matter;
- V1 (0.25% $CuSO_4$ solution) – 6.46 g% dry matter;
- V2 (0.10% $CuSO_4$ solution) – 6.36 g% dry matter.

Samples V1 – 0.10% $CuSO_4$ solution and V1 – 0.15% $CuSO_4$ solution indicated lower values in comparison to the control sample (6.27 g% dry matter) of 4.96 g% and 6.26 g% dry matter (figure 1). On V2 (watering with 2 ml of tap water and spraying with 2 ml of copper sulfate solution), the dry matter content grows simultaneously with the increase in the concentration of micro-elements (figure 1). High values were registered for variants V2 –0.15% $CuSO_4$ solution (10.32 g% dry matter) and V2 – 0.25% $CuSO_4$ solution (11.27 g% dry matter).

A closer value to the one of the control sample in mixed treatment (watering and spraying with copper sulfate) was registered by V3 – 0.10% CuSO4 solution (8.70 g% dry matter), the other samples, V3 – 0.15% $CuSO_4$ solution and V3 – 0.25% $CuSO_4$ solution, recording values which were 13 times higher, that is of 17 %.

We observe the beneficial effects of dry matter storage in vegetal material on variants V2 and V3 along with the increase in the concentration of copper sulfate solution.

The water content in the vegetal material of *Calendula officinalis* varied from 76.46 g% (V3 – 0.25% $CuSO_4$ solution) to 95.04 g% (V1 –0.10% CuSO4 solution) (Fig. 1).

Due to the interdependence of the two characters (dry matter and water content), calendula samples showed a rich dry matter content and low water content and vice versa.

Most samples showed water content values of over 80%. The increasing concentration of copper sulfate decreased water concentration, and V1 treatment stimulated the accumulation of water in plant tissues (Fig. 1.).

The content of chlorophylls *a* and *b* indicated an upward trend compared to control samples. The content of chlorophyll *a* ranged from 0.321 mg/g (control sample) and 1.101 mg/g (sample V3 -0.25% CuSO4 solution). Most of the samples after treatment with copper sulphate solution showed values above 0.250 mg/g (V1 and V2), and samples treated by irrigation and spraying with copper sulphate solution showed the highest values of over 0.800 mg/g (Fig. 2.).

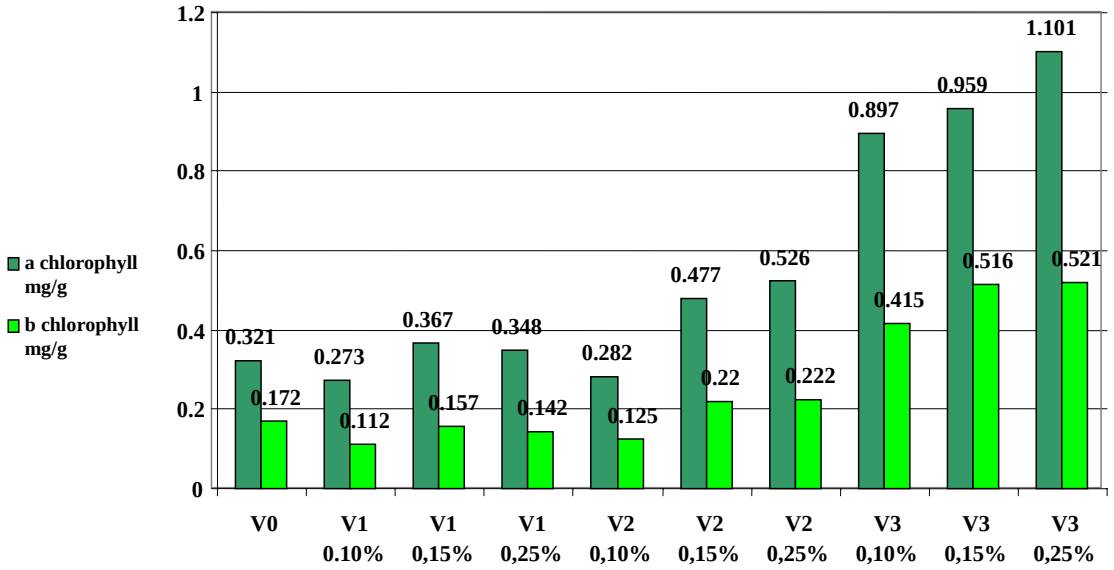

Fig. 2. Dynamics of chlorophyll *a* and *b* in *Calendula officinalis* L. plants treated with a $CuSO_4$ solution in different concentrations

The measurements carried out by treatment with an aqueous solution of copper sulfate show the increase in chlorophyll *a* content, as long as the concentration of copper sulfate is increased; thus, the values recorded were:

- V1 (0.15% $CuSO_4$ solution) - 0.348

mg/g chlorophyll *a*;

- V1 (0.25% $CuSO_4$ solution) – 0.526 mg/g chlorophyll *a*;
- V2 (0.10% $CuSO_4$ solution) – 1.101 mg/g chlorophyll *a*.

Concentrations of 0.10% copper sulfate on variants V1 and V2 did not stimulate chlorophyll biosynthesis in the green parts of the plant, recording lower values of 0.048 or 0.039 versus the control.
Higher values of chlorophyll *a*content are recorded for all variants, but at concentrations of 0.15%, 0.25% and V3 0.10% copper sulphate V3 (Fig. 2).

Chlorophyll b registers the same ascending trend of biosynthesis for variants V2 and V3 at concentrations of 0.15%, 0.25% copper sulfate, 0.10% for V3.

The content of chlorophyll *b* ranged between 0.172 mg/g (control sample) and 0.521 mg/g (sample V3 – 0.25% $CuSO_4$ solution) (Fig. 2).

On the average, the content of chlorophyll *b* registers values:

- over 0.100 mg/g (V0, V1 and V2 – 0.10% $CuSO_4$ solution);
- over 0.220 mg/g (V2 – 0.15% and 0.25% $CuSO_4$ solution);
- over 0.410 mg/g (V3).

Root length character values were between 1.8 - 28.9 cm. The arithmetic mean of the values obtained by measuring roots in control sample was 4.96 cm, 8.71 lower than sample V1 - 0.25% $CuSO_4$ solution (Fig. 3).

For root length character, we can observe a positive influence on its growth after treatment with copper sulphate; recorded values exceed 5 cm in most samples (Fig. 3).

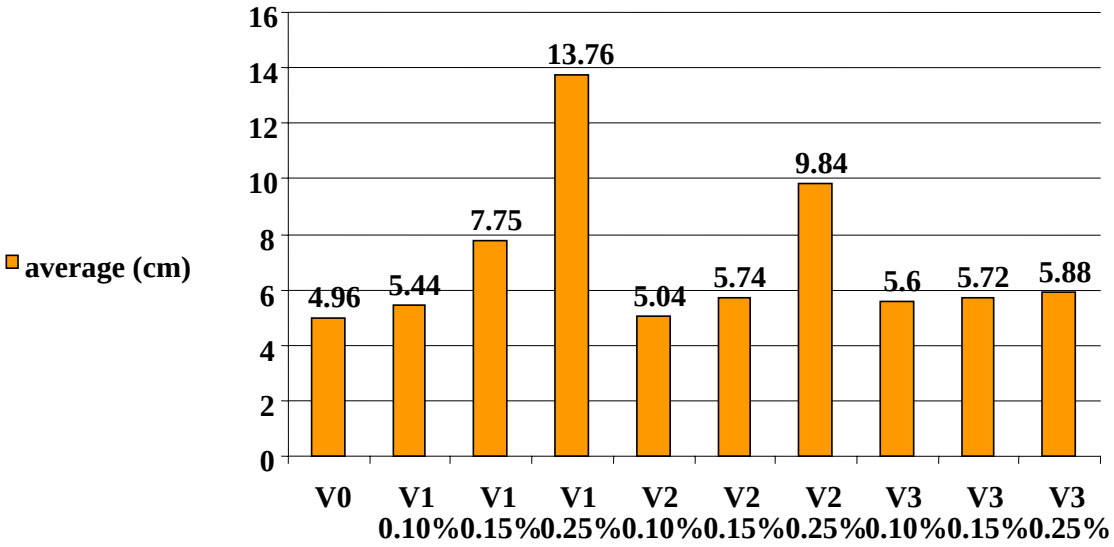

Fig. 3. Variation of the root length character in *Calendula officinalis* L. treated with a $CuSO_4$ solution in different concentrations

Values close to the root length character were recorded for samples: V1 –0.10% $CuSO_4$ solution (5.44 cm), V2 – 0.15% $CuSO_4$ solution (5.74 cm), V3 – $CuSO_4$ solutions of 0.10%, 0.15%, 0.25% (5.6, 5.72, respectively 5.88 cm).

Variant V1, watered with 0.25% $CuSO_4$ solution, manifested a positive effect on calendula root growth, which registered the maximum value of 13.76 cm. The same situation is met for variant V2 – sprayed with 0.25% $CuSO_4$ solution – 9.84 cm (Fig. 3.).

For stem length character, we can notice a slight inhibition of hypocotyl growth and stem elongation, considering that, after treatment, all samples showed lower values than the control sample (3.8 cm) (Fig. 4.).

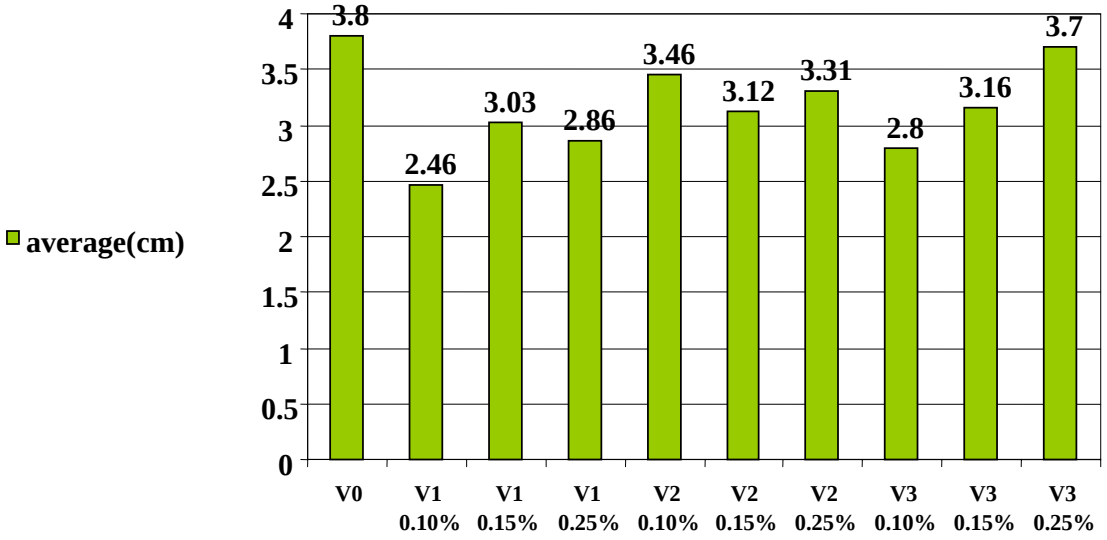

Fig. 4. Variation of the hypocotyl length character in *Calendula officinalis L.* plants treated with a $CuSO_4$ solution in different concentrations

The average values recorded are around 3 cm, only variant V3 - 0.25% $CuSO_4$ solution being closer to the control value, with a stem and hypocotyl length of 3.7 cm. The combined treatment and the greater amount of copper had a positive impact on the growth of these plant organs (Fig. 4).

The cotyledon length character indicated values ranging from 2.33 cm (V0) to 5.26 cm (V1 - 0.10% $CuSO_4$ solution). Variants V1 - 0.10% $CuSO_4$ solution and V2 - 0.25% $CuSO_4$ solution had the highest values of this biometric character (5.26 and 4.21 cm) (Fig. 5).

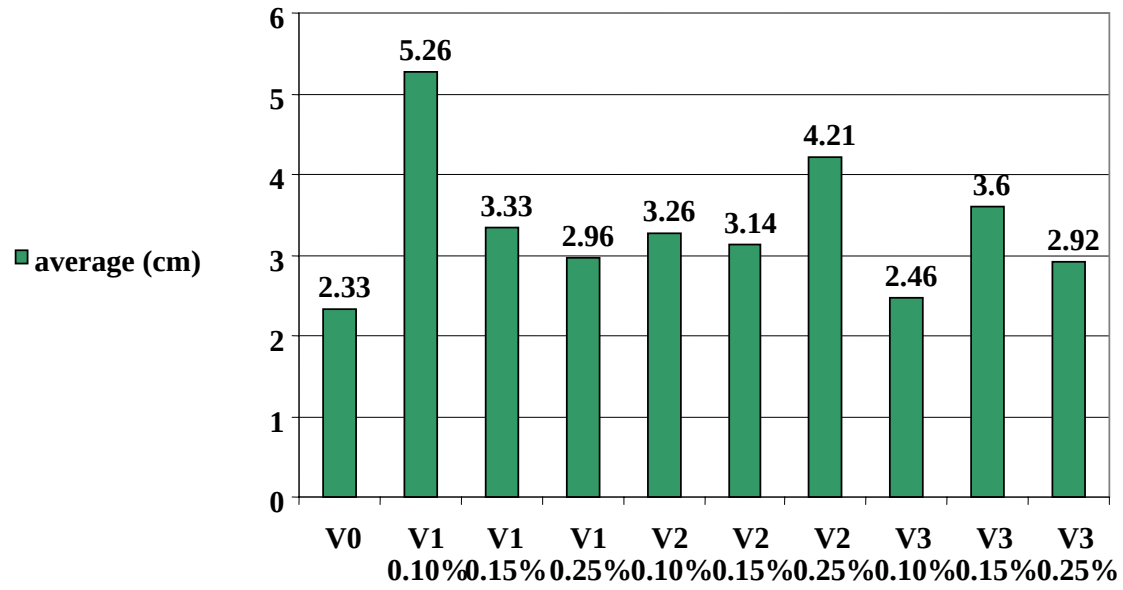

Fig. 5. Variation of the cotyledon length character in *Calendula officinalis L.* plants treated with a $CuSO_4$ solution in different concentrations

All samples have a positive influence on the growth of cotyledons, on their length compared with control samples, varying between 0.13 and 2.93 cm.
Closer values over 3 cm were indicated for: V1 - 0.15% $CuSO_4$ solution (3, 33 cm), V2 - 0.10% $CuSO_4$ solution (3.26 cm), V2 - 0.15% $CuSO_4$ solution (3.14 cm) and V3 - 0.15 % $CuSO_4$ solution (3.6 cm).

We conclude that the treatment with copper sulfate stimulates the growth of roots and cotyledons, but inhibits the growth of stem and hypocotyl.

CONCLUSIONS

The treatment of plants for 4 days with an aqueous solution of copper sulfate favored the accumulation of dry matter on the variants V2 and V3 and of the water content - V1.

The content of chlorophyll *a* and *b* recorded increasing values for variants V2 and V3 compared with control samples.

Copper sulfate treatment stimulates root growth, cotyledon elongation but inhibits the growth of hypocotyl and stem.

The root length character showed average values over 5 cm in most samples, regardless of the treatment methods used.

The hypocotyl and stem length character for the treated samples showed lower values than control samples (below 3.8 cm).

The cotyledon length character after treatment with copper sulphate recorded higher values of 0.13 to 2.93 cm than the control sample.

We recommend the application of treatment with copper sulfate solution by spraying (V2) and mixed method (V3) on these variants, due to their high content of dry matter and water, chlorophyll *a* and *b* as well as vegetative growth.

ABSTRACT

The study presents the results obtained in testing the moderate application of copper sulfate on calendula plants, in comparison to the high quantity commonly used for different species of plants cultivated in private gardens.

As a result of our research, we can prove the positive effect of copper sulfate in the storage of dry matter and assimilating pigments in vegetal tissues for tested variants, as well as the existence of biometric growths.

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