THE INFLUENCE OF SOME MICROELEMENTS ON GERMINATION AND CHLOROPHYLL QUANTITY IN *OCIMUM BASILICUM L.*

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Abstract

In Cormophyta plants the process of mineral nutrition takes places through the root system and through leaves. This process is a physiologic feeding process with nutritive substances for the plants.

In this paper authors studied the influence of some microelements: Mg, Pb and Zn on the Ocimum basilicum L. seeds germination and chlorophyll quantity.

Ocimum basilicum L. is used in phytopherapy, because many beneficial effects. Being an annualy cultivated plant, it is important to choose the proper cultivation considering all environmental factors.

We observed that the studied metals have multiple influences, such as: zinc has high toxicity, inhibits the seed germination, even if administered in low concentrations; lead is toxic in high concentrations, but in low concentration we observed the seed germination; magnezium has a stimulating effect on the basil seeds germination. The amount of chlorophyll decreases under the action of the studied metals , however there is a slight increase in the amount of chlorophyll b. It was also noticed that there is no significantly effect on the total amount of chlorophyll pigments.

Keywords: Ocimum basilicum, microelements, chloroplyll quantity

INTRODUCTION

The *Ocimum* genus includes more than 150 species. However, some attributions are difficult, due to the interference of man with selection, cultivation and hybridization within the genus and to large morphological variation among the different species (Pallag A., 2015). A system of standardized descriptions, based on volatile oils, has been proposed, but its use is limited by the fact that several environmental factors may influence the plant chemical composition (Labra M et al, 2004, Ciocârlan V., 2000).

Ocimum basilicum L., basil, of the Family Lamiaceae, sometimes known as Saint Joseph's Wort in some English-speaking countries. It is a delicate low-growing herb, originally native in tropical Asia. It is an annualy cultivated plant in Romania.

It grows between 20 - 60 cm tall, with opposite, light green, silky leaves of 1.5 - 5 cm long and 1 - 3 cm wide. It tastes somehow like anise, with a strong, pungent, sweet smell.

Basil is very sensitive to cold, with best growth in hot, dry conditions (Bilal et al, 2012, Saha S. et al, 2012, Pallag A., 2015, Cicarlan V., 2000).

Quantitative proportion of the chemical elements in plant's body vary, and are conventionally divided into macro elements, microelements and ultra-microelements. Microelements are present in small quantities in plant's body, but their presence is indispensable.

The most important microelements are: Fe, Mn, B, Sr, Cu, Zn, Ba, Li, I, Br, Al, Ni, Mo, Cd, As, Pb, Va, Rb, being present in amounts ranging between 0.00001 and 0.001% of dry plant. They are found in general metabolism, in plant growth and development, in the immune processes (Chaney, R.L. et al 2000, Pallag A., 2007).

Lack of microelements can lead to physiological diseases accompanied by slowing or cesation of root, stem, leaves and fruits growth. Excessive increase in the quantities of these substances in soil, mainly due to pollution also leads to metabolic disorders in plants, but also affects the quality of the food that may be harmful to health (Baker, A.J.M., P.L. Walker., 1990, Bathory D. et al 1997, Baker, A.J.M et al., 2000).

The uptake and distribution of microelements and metals were studied with great attention in crops due to the power of their importance (Pallag Annamaria et al., 2006, Şumălan Radu, 2006, Țiț M. et al, 2012).

The distribution of metals in plants depends on the concentration, type of metal and the plant (Bungău S. et al., 2011). Most of the time the highest concentration was found in the roots, because they are in direct contact with the toxic environment.

The influence of microelements in plant's life is present in all biological levels. (Assche, F., 1990, Bungău S., 2014, Nanthi S. et al. 2003, Gâdra Ștefania, 2009).

MATERIAL AND METHODS

The preparation of biological material

To establish the effect of microelements on germination the "*Constantinescu compared test*" bioassay was used (Agrios G. N., 1978).

We put for germination lots of 20 *Ocimum basilicum L.* seeds in Petri dishes on filter paper soaked with common water boiled and cooled. The seeds were kept 24 hours in darkness and temperatures of 20-24 ^{0}C, during which they germinates and the root go to a maximum of 1-2 mm length.

The preparation of test solutions:

All the solutions $Pb(NO_3)_2$, $ZnSO_4$, $MgSO_4$ were prepared at concentration of 2%; 1%; 0.5%; 0.25% with distilled and demineralized water.

In groups the germination occurred, 1 g of the newly formed leaves were grinded in mortar with 10-15 mL 70% ethanol, then filtered. The filtrates represent the samples for chlorophyll quntity determination.

All the determination were made with a spectrophotometer and alcohol was used as the control sample. We measured the absorbance at 646.6 nm wavelength (the wavelength at which absorption is maximum for *chlorophyll b*, respectively 663.6 nm wavelength - the wavelength at which absorption is maximum for *chlorophyll a)*.

RESULTS AND DISSCUTIONS

It was observed the influence of the elements mentioned above in seed germination of *Ocimum basilicum L.* We organized the study groups, which we treated with different solutions, of different concentrations, of $Pb(NO_3)_2$, $ZnSO_4$ and $MgSO_4$ (Figure 1 and 2).

In Table 1 are presented the results of germination after three days, and in Table 2 - after 7 days. It is noted that after three days, the seeds treated with $Pb(NO_3)_2$ and $ZnSO_4$ do not germinate and those treated with $MgSO_4$ germinates very slow, compared to the control group. The stage of the seeds'germination is shown in Figure 3 and 4.

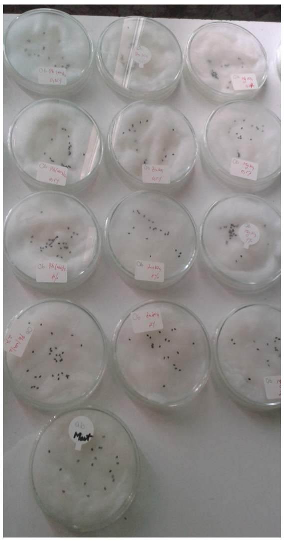

Fig.1. The organization of the study groups

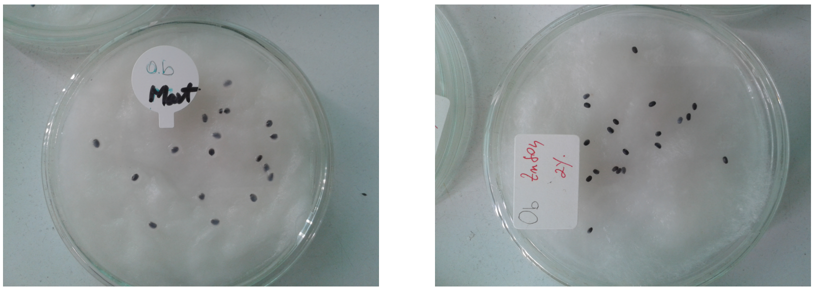

Fig. 2. The control group and the $ZnSO_4$ 2% group

Table 1.

The percentage of the seeds that germinated after 72 hours (3 days)

Used concentrations %	Mg %	Pb %	Zn %	
2.00	5	0	0	Control 25%
1.00	5	0	0	
0.50	10	0	0	
0.25	15	0	0	

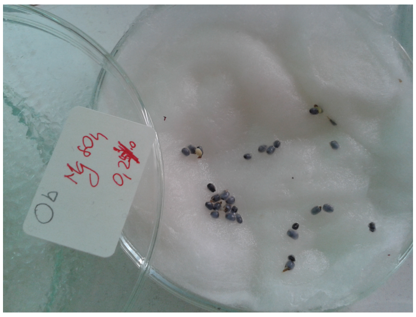

Fig. 3. $MgSO_4$ 0.25% group, after 3 days

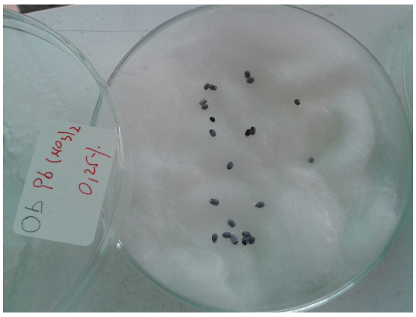

Fig.4. $Pb(NO_3)_2$ 0.25% group, after 3 days

Table 2.

The percentage of the seeds that germinated after 7 days

Used concentrations %	Mg %	Pb %	Zn %	
2,00	5	0	0	Control 50%
1,00	25	10	0	
0,50	40	10	0	
0,25	55	25	0	

After 7 days it was observed that seeds treated with $ZnSO_4$ do not germinate at all, those treated with $Pb(NO_3)_2$ at concentrations greater than 2% do not germinate and at lower concentrations germinate very little.

The best results are obtained when $MgSO_4$ (at the concentration of 0.25%) determines the germination of a larger number of seeds than in the control group (Figure 5, 6, 7, 8).

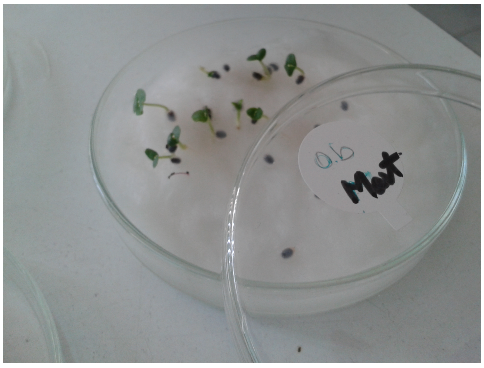

Fig 5. Control group, after 7 days

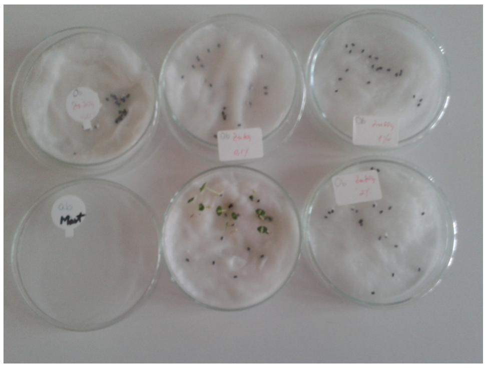

Fig.6. $ZnSO_4$ group, after 7 days

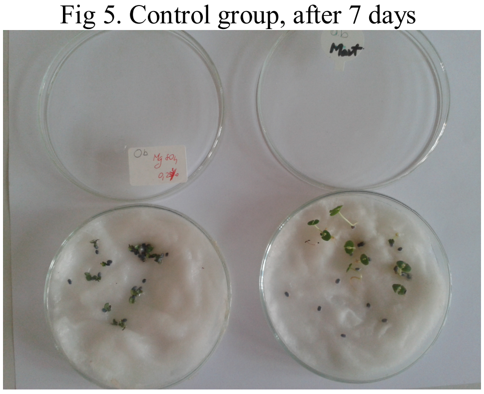

Fig.7. $MgSO_4$ group, after 7 days

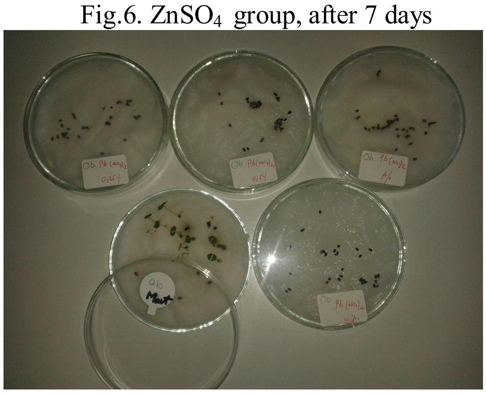

Fig.8. $Pb(NO_3)_2$ group, after 7 days

The influence of microelements on the amount of chlorophyll

We obtained a mixture of chlorophyll pigments from the leaves of *Ocimum basilicum L.*, from different lots, in which the percentage of germinated seeds was min. 20%, after 7 days.

- Group treated with the solution of $MgSO_4$ 0.25%;
- Group treated with the solution of $MgSO_4$ 0.50%;
- Group treated with the solution of $MgSO_4$ 1.00%;
- Group treated with the solution of $Pb(NO_3)_2$ 0.25%;
- Control group.

From these groups, there were taken 1 g of leaves, were grinded in the mortar with 10-15 mL 70% ethanol, then filtered. The filtrates represents the samples. The chlorophyll quantity from the samples was calculated using the formulas of some authors (Porra, 2002, Azervedo R.A., Lea P.I., 2005, Gâdra Ștefania, 2009).

$$[Chl\ a]=12.25\ E663.6–2.55\ E646.6\ \mu g/mL$$
$$[Chl\ b]=20.31\ E\ 646.6–4.91\ E663.6\ \mu g/mL$$
$$[Chl\ a+b]=17.76\ E\ 646.6+7.34\ E\ 663.6\ \mu g/mL$$
$$[Chl\ a/b]=12.25\ E663.6–2.55\ E646.6/20.31\ E\ 646.6–4.91\ E663.6\ \mu g/mL$$

The obtained results were presented in Table 3, 4 and in Figure 9.

Table 3.

Obtained absorbances

Nr crt	**Lot of study**	**Absorbances at 646.6 nm**	**Absorbances at 663.6 nm**
1	$MgSO_4$ 0.25%	1.4340	1.2980
2	$MgSO_4$ 0.50%	1.4010	1.1850
3	$MgSO_4$ 1.00%	1.2250	0.8060
4	$Pb(NO_3)_2$ 0.25%	1.1480	0.4320
5	Control	1.2570	1.0610

Table 4.

Concentrations of chlorophyll a and b under the influences of the metal solutions

Group	$MgSO_4$ 0.25%	$MgSO_4$ 0.50%	$MgSO_4$ 1.00%	$Pb(NO_3)_2$ 0.25%	Control
[Chl a]	12.24 µg/mL	10.94 µg/mL	6.74 µg/mL	2.36 µg/mL	9.79 µg/mL
[Chl b]	22.75 µg/mL	22.63 µg/mL	20.92 µg/mL	21.19 µg/mL	20.32 µg/mL
[Chl a+b]	34.99 µg/mL	33.57 µg/mL	27.66 µg/mL	23.55 µg/mL	30.11 µg/mL
[Chl a/b]	0.53 µg/mL	0.48 µg/mL	0.32 µg/mL	0.11 µg/mL	0.48 µg/mL

Under the action of $MgSO_4$ 1.00% and $Pb(NO_3)_2$ 0.25% the amount of chlorophyll decreases, however there is a slight increase in the amount of chlorophyll b, compared to the control sample. Under the action of $MgSO_4$ 0.25% and $MgSO_4$ 0.50% the amount of chlorophyll a and b increases, compared to the control sample.

Not significantly effect on the total amount of chlorophyll pigments (a and b) was observed in the case of using $Pb(NO_3)_2$ 0.25%.

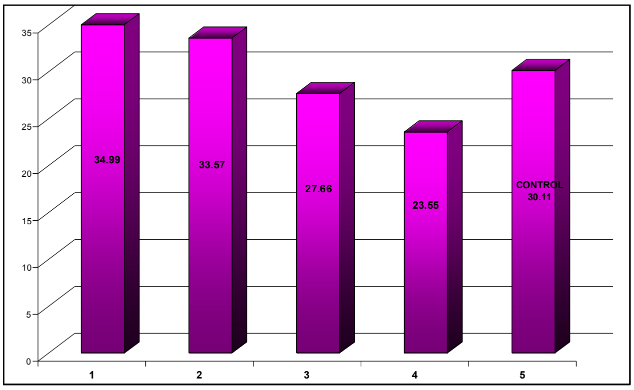

Fig.9. Total quantity of chlorophylic pigments (μg/mL) in the case of using 1.$MgSO_4$ 0.25%, 2.$MgSO_4$ 0.50%, .$MgSO_4$ 1.00%, 4.$Pb(NO_3)$ 0.25%, 5.Control

The ratio of the amount of chlorophyll a and chlorophyll b significantly decreased under the action of $MgSO_4$ 1.00% and $Pb(NO_3)_2$ 0.25%.

CONCLUSIONS

The influence of metals on the germination process varies depending on the species, the time on which the seeds were soaked in the studied metal's solutions. The germination process underwent a profound morphological change in the test conducted. The solutions tested at different concentrations caused distortions in the roots, on their viability.

High concentrated solution of 2% and 1%, caused specifically inhibition of the germination of all the elements under study. In concentrations of 2%, in contact just with the Mg sollutions the germination occured, but in very low percent - 5%. The concentration of 0.5% and 0.25% Zn inhibited the germination with percentage of 100%.

Among the metals studied, Zn has high toxicity, even if administered in low concentrations.

Lead is toxic in high concentrations and less toxic in low concentrations, it is affecting the germination process and the amount of chlorophyll. Magnesium in concentrations lower than 0.25%, has stimulated the gemination, so the seeds germinated in a percentage higher than 5% compared to the control group; at the same time the levels of chlorophyll increased in the amount of germinated plants.

The basil is a species widely used both as a spice and in phytotherapy, too. Being a plant annually grown in our country it has major importance choosing the proper cultivation conditions, taking into account all environmental factors.

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