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Effects of Molybdenum Treatments on Maize and Sunflower Seedlings

E. Bodi, Sz. Veres, F. Garousi, Sz. Varallyay, B. Kovacs

Abstract—The aim of the present study was to examine whether increasing molybdenum (Mo) concentration affects the growth and Mo concentration of maize (*Zea mays* L. cv Norma SC) and sunflower (*Helianthus annuus* L. cv Arena PR) seedlings within laboratory conditions.

In this experiment, calcareous chernozem soil was used and Mo was supplemented into the soil as ammonium molybdate $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}]$ in four different concentrations as follow: 0 (control), 30, 90 and 270 $\text{mg}\cdot\text{kg}^{-1}$.

In this study, we found that molybdenum in small amount (30 $\text{mg}\cdot\text{kg}^{-1}$) affects positively on growth of maize and sunflower seedlings, however, higher concentration of Mo reduces the dry weights of shoots and roots. In the case of maize the highest Mo treatment (270 $\text{mg}\cdot\text{kg}^{-1}$) and in sunflower 90 $\text{mg}\cdot\text{kg}^{-1}$ treatment caused significant reduction in plant growth.

In addition, we observed that molybdenum contents in the roots and shoots were very low in case of control soil but were significantly elevated with increasing concentration of Mo treatment. Only in case of sunflower the highest 270 $\text{mg}\cdot\text{kg}^{-1}$ Mo treatment caused decrease in Mo concentration.

Keywords—Dry weight, maize, molybdenum, sunflower.

I. INTRODUCTION

MOLIBDENUM is a microelement found in the soil and is needed for the growth of biological organisms which includes the plants [1]. This necessary nature of Mo was first shown by [2]. Plants which are grown in a purified solution cultures shown deficiency symptoms, because of the lack of molybdenum, and symptoms were prevented by adding 0.01 $\text{mg}\cdot\text{L}^{-1}$ Mo to the root. Normal growth was restored to deficient plants if molybdenum was placed onto the foliage, therefore showing that molybdenum has its effect directly on the growth and not indirectly by changing the root environment [3].

The total amount of Mo in agriculture soils averages 1-2 $\text{mg}\cdot\text{kg}^{-1}$ [4], [5]. This is stated by [6], that in soils Mo happens to be in the four major forms: (1) water-soluble present in the soil solution, (2) Mo occluded with oxides, for example aluminum oxide and iron oxide, (3) bound in organic matter and (4) solid phases like molybdenite (MoS_2), powellite (CaMo_4), ferrimolybdenite ($\text{Fe}_2(\text{MoO}_4)$), wulfenite (PbMoO_4).

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The most effective way for plants is to take it as a molybdate ion (MoO_4^{2-}). Once molybdate anion enters into the roots, it stays biologically inactive until it is joined by a pterin compound called molybdenum cofactor (Moco) [7], [8].

There is a direct availability of molybdenum for plants to the pH of the soil, concentration of adsorbing oxides like iron oxides, amount of water drainage, and organic compounds found in the soil colloids. Mo deficiency in plants can happen in soils (1) with low total Mo, (2) where Mo is isolated by oxihydroxides, (3) in extensively weathered soils, (4) when pH value is lower than 6, or (5) in sandy well drained soils [4]. The opposite is true when higher levels of Mo can be found in alkaline soils, soils rich with organic matters also under wet conditions [9]. Several research studies confirm that the presence of large quantities of Mo in plants does not produce harmful effects or cause significant reductions in the crops. Animals however are sensitive to a high-Mo forage ration, which could lead to Mo-induced Cu deficiency [10].

Conversely, plants are more sensitive to molybdenum deficiency than to toxicity. Deficiency symptoms for most micronutrients appear on the young leaves at the top of the plant because most micronutrients are not easily translocated. Mo is an exception that is easily translocated and its deficiency symptoms appear on the entire plant, but the symptoms normally show themselves as a nitrogen shortage, especially seen in legumes. These symptoms are connected to the function of molybdenum in nitrogen metabolism, for example its part on N_2 fixation and nitrate reduction. However, plants suffering from great deficiency often show symptoms that are related only to molybdenum [11].

For instance, molybdenum deficient leaf edges tend to roll up. Marginal chlorosis of older leaves may happen. In many occasions, necrosis follows and the whole plant is stunted. Young leaves become mottled and their leaf edges are very narrow. In addition, molybdenum deficiency reduces photosynthesis, lower sugar and vitamin C contents. Vitamin C content of leaves can decrease by 25% compare to the normal level. Affected plants gather nitrate in their leaves. Protein content of the plants also reduces [12]-[14].

Mo deficiency also has great effects on the growth of plants [15], [1]. Agarwala et al. [16] saw that in Mo-deficient plants, low growth and a reduction in leaf size are very common and in dicotyledonous species much more reduction in size can be observed. Some literature established Mo application can affect growth stimulation on acidic mineral soils [17], [18].

The aim of present study was to examine whether molybdenum treatments effects on the growth and uptake of molybdenum of maize and sunflower seedlings. Therefore, dry

weights and Mo concentration of shoots and roots of our seedlings were estimated.

II. MATERIALS AND METHODS

A. Cultivation in Rhizoboxes

In this experiment calcareous chernozem soil was taken from Látókép Experimental Station of University of Debrecen.

Composition of the applied soil can be seen in Table I.

Molybdenum was added to the soil as ammonium molybdate $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}]$ in four different concentrations as follows: 0 (control), 30, 90 and 270 $\text{mg}\cdot\text{kg}^{-1}$. Then, soil was placed in the rhizoboxes. The rhizobox, that was used, is a rectangular, 23.5 cm x 10 cm x 1 cm sized, transparent on one side and plastic which allows studying the growth of roots. In order to ensure steady water uptake by plants, moist filter papers were placed at the bottom of rhizoboxes before adding the soil.

TABLE I
PARAMETERS OF SOIL APPLIED IN THE EXPERIMENTS CARRIED OUT IN
RHIZOBXES

Depth	0-0.3 m
pH (KCl)	5.71
pH (H ₂ O)	6.58
Soil texture category	loamy clay
Total water-soluble salt	0.015%
CaCO ₃	0.202%
Humus	3.54%
KCl-soluble NO ₃ -N+NO ₂ -N	8.04
AL-soluble P ₂ O ₅	199 $\text{mg}\cdot\text{kg}^{-1}$
AL-soluble K ₂ O	451 $\text{mg}\cdot\text{kg}^{-1}$
AL-soluble Na	332 $\text{mg}\cdot\text{kg}^{-1}$
KCl-soluble Mg	176 $\text{mg}\cdot\text{kg}^{-1}$
KCl-soluble SO ₄ ²⁻ -S	6.04 $\text{mg}\cdot\text{kg}^{-1}$
KCl-EDTA-soluble Cu	5.79 $\text{mg}\cdot\text{kg}^{-1}$
KCl-EDTA-soluble Zn	7.9 $\text{mg}\cdot\text{kg}^{-1}$
KCl-EDTA-soluble Mn	262 $\text{mg}\cdot\text{kg}^{-1}$

Since maize (*Zea mays* L. cv Norma SC) is monocotyledon and sunflower (*Helianthus annuus* L. cv Arena PR) is dicotyledon, they were chosen for rhizoboxes research. We considered it is important that the experiment was performed with monocotyledon and dicotyledon plants, too because two types of plants have numerous differences in the uptaking mechanism of nutrients [19].

Surface of seeds was sterilized by 18% H₂O₂ solution for 25 minutes. Disinfected seeds were rinsed several times with ionized water then were soaked with 10 mM CaSO₄ solution for 4 hours to get better germination.

Disinfected maize and sunflower seeds were geotropically germinated between moist filter papers in 24°C in the climate room, then uniform and best-developed seedlings were transferred into rhizoboxes. Three seedlings were planted in every rhizoboxes and each treatment had five replicates.

After planting the seedlings in the soil, the transparent side walls of rhizoboxes were covered with black foil. Plants were geotropically stimulated to force root growth along the

transparent wall of the box thus, allowed us convenient monitoring of the roots.

Rhizobox experiments were carried out in a climate room of Institute of Crop Sciences, Department of Agricultural Botany, Crop Physiology and Biotechnology with the following controlled environmental conditions: a 16-h light period day with a light intensity of 300 $\mu\text{molm}^{-2}\text{s}^{-1}$, at 25/20°C day/night temperatures and relative humidity were maintained between 65-75%.

The experiment with maize ended 9 days after planting and the experiments with sunflower were evaluated 12 days after planting.

B. Sample Preparation and Analytical Methods

At the end of the experiments, shoots were separated from roots. Plant parts were dried at 85°C until constant weight was achieved then, cooled to room temperature and weighed by an analytical scale (OHAUS).

Wet digestion method with nitric acid and hydrogen peroxide was applied during sample preparation of plants [20]. 0.5 g sample was measured. After adding 5 cm³ concentrated HNO₃ (65 m/m%, Scharlau Chemie, Spain) they were predigested for a night, then heated to 60°C for 45 min in a LABOR MIM OE 718/A block digestion instrument. Following the first digestion step, 1.5 cm³ 30% H₂O₂ (Darmstadt, Merck, Germany) was added to the samples, and digestion was continued at 120°C for another 90 min. After cooling the samples to room temperature, volume was adjusted to 25 cm³ with deionized water. Then samples were mixed by shaking and filtered through FILTRAK 388 filter paper. Also blank experiment was done.

Element analysis was carried out by inductively coupled plasma optical emission spectrometry (ICP-OES) using a Perkin Elmer OPTIMA 3300 DV type analytical instrument [21].

C. Statistical Analysis

For the statistical analysis we used One-Way analysis of variance (ANOVA) and Duncan's test. The significance was evaluated at the P<0.05 level. The statistical analyses were carried out using SPSS v.17.0 software.

III. RESULTS

A. Dry Weights of Maize and Sunflower Seedlings

The results of our research on dry weight of maize seedlings are summarized in Table II. The average dry weights of shoots and roots were 0.0238±0.0034 g and 0.0400±0.0098 g, respectively, in the case of control treatment. In the presence of 30 and 90 $\text{mg}\cdot\text{kg}^{-1}$ molybdenum a slight increase in dry weight of shoot was found compared to the control seedling.

However, the highest Mo concentration resulted in reduction in dry weights of shoots about 23%, in comparison with control levels.

Although in case of root, the 30 and 90 $\text{mg}\cdot\text{kg}^{-1}$ molybdenum treatments caused a small increase in dry weight, it did not show a significant difference between the results.

Our data show that only the largest treatment resulted in a significant reduction.

The dry weight of sunflower shows significantly different results compared to the maize (Table III), which due to the two types of nutrients uptake mechanism of examined plants.

TABLE II
THE EFFECT OF MOLIBDENUM TREATMENTS ON DRY WEIGHT OF MAIZE SEEDLINGS

Mo-treatment (mg kg ⁻¹)	Dry weight of shoot (g plant ⁻¹)	Dry weight of root (g plant ⁻¹)
control	0.0238 ^{ab} ±0.0034	0.0400 ^b ±0.0098
30	0.0267 ^{bc} ±0.0046	0.0394 ^b ±0.0103
90	0.0308 ^b ±0.0083	0.0374 ^b ±0.0120
270	0.0210 ^a ±0.0049	0.0305 ^a ±0.0069

Values are expressed as mean ± s.e.; n=5

TABLE III
THE EFFECT OF MOLIBDENUM TREATMENTS ON DRY WEIGHT OF SUNFLOWER SEEDLINGS

Mo-treatment (mg kg ⁻¹)	Dry weight of shoot (g plant ⁻¹)	Dry weight of root (g plant ⁻¹)
control	0.0531 ^b ±0.0112	0.0201 ^c ±0.0069
30	0.0608 ^c ±0.0086	0.0255 ^d ±0.0053
90	0.0379 ^a ±0.0088	0.0156 ^b ±0.0046
270	0.0347 ^a ±0.0058	0.0115 ^a ±0.0028

Values are expressed as mean ± s.e.; n=5

Only 30 mg·kg⁻¹ treatment caused significant increase in growth of sunflower seedlings. We can state dry weights increased in shoots by 15% and in roots by 26%, as compared to the control.

In contrast, 90 and 270 mg·kg⁻¹ inhibited significantly the growth of shoots and roots.

B. Molybdenum Concentrations of Maize and Sunflower Seedlings

The addition of Mo to the soil resulted significant increase of Mo concentration in tissues of maize seedlings (Fig. 1). A greater amount of Mo was found in roots than in shoots in the case of all treatments. Mo concentration of root in the case 30 and 90 mg·kg⁻¹ treatments is nearly twice and in the case of 270 mg·kg⁻¹ treatment was approximately three times Mo concentration of shoot.

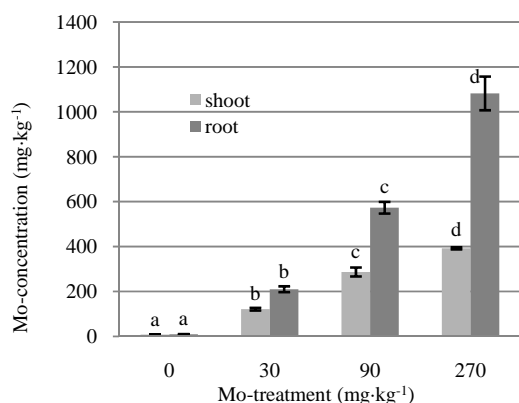


Fig. 1 Molybdenum (Mo) concentration (mg·kg⁻¹) of maize seedlings' shoot and root depends on molybdenum treatments (0, 30, 90 and 270 mg·kg⁻¹), (n=3±s.e.)

In Fig. 2 changes of Mo concentration in shoots and roots of maize seedlings depend on Mo treatment that has been shown. The content of Mo in seedlings changed in the presence of Mo compared to the control. The level of Mo content in the case 30 and 90 mg·kg⁻¹ Mo increased 40.9- and 135-fold in shoots, as well as 68.3- and 146-fold in roots, respectively, in comparison with control but it should be emphasized that the highest Mo concentration caused a slight decrease of Mo accumulation in seedlings compared to the 90 mg·kg⁻¹ treatment.

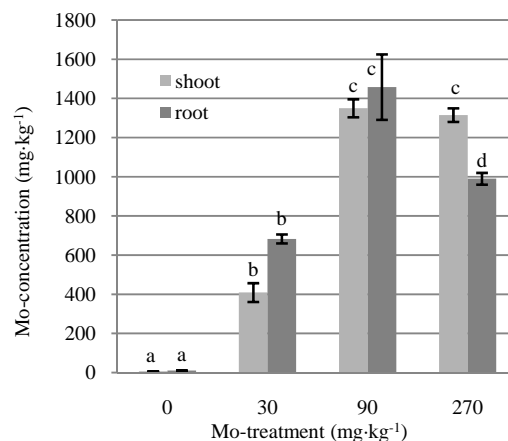


Fig. 2 Molybdenum (Mo) concentration (mg·kg⁻¹) of sunflower seedlings' shoot and root depends on molybdenum treatments (0, 30, 90 and 270 mg·kg⁻¹), (n=3±s.e.)

IV. CONCLUSIONS

In this study we found that molybdenum in small amount (30 mg·kg⁻¹) affects positively on growth of maize and sunflower seedlings, however, further increase of Mo content reduced the dry weights of shoots and roots. In case of maize the highest Mo treatment (270 mg·kg⁻¹) and in case of sunflower 90 mg·kg⁻¹ treatment caused a significant reduction in plant growth.

In addition, we observed that molybdenum concentration in maize and sunflower seedlings increased due to molybdenum treatment. Only in case of sunflower the highest (270 mg·kg⁻¹) Mo treatment caused decrease in Mo concentration.

By examining roots and shoots of experimental plants separately, we found higher molybdenum content in roots than in shoots however, only in the case of the largest sunflower treatment resulted higher Mo concentration in the shoot.

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