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THE EFFECTS OF Mo, Cu, Mn ON SOME PHYSIOLOGICAL INDEXES OF COMMON BEAN VARIETY NHPO4 AT SEEDLING STAGE

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ABSTRACT

This paper presents the results about effect of Mo, Cu, Mn to some physiological indexes of common bean variety NHP04 at seedling stage. The results showed that Mo, Cu and Mn affected water content in leaves, water holding capacity, transpiration intensity and chlorophyll content in leaves, in which Mo had the most effect. The results of this research can serve as scientific basis to enhance the tolerance and productivity of the common bean varieties and the plant.

Keywords: common bean, micro-nutrients, physiological indexes, seedling stage.

TÓM TẮT

Ảnh hưởng của Mo, Cu, Mn đến một số chỉ tiêu sinh lí của giống đậu cô ve NHPO4 ở giai đoạn cây con

Bài báo này, trình bày kết quả nghiên cứu ảnh hưởng của một số nguyên tố vi lượng đến một số chỉ tiêu sinh lí của giống đậu cô ve NHPO4 ở giai đoạn cây con. Kết quả cho thấy Mo, Cu, Mn đều ảnh hưởng đến hàm lượng nước trong lá, khả năng giữ nước của lá, cường độ thoát hơi nước và hàm lượng diệp lục tổng số trong lá; trong đó, Mo có ảnh hưởng rõ rệt nhất. Kết quả nghiên cứu là cơ sở khoa học đưa ra các giải pháp nhằm nâng cao khả năng chống chịu và năng suất của các giống đậu cô ve nói riêng và cây trồng nói chung.

Từ khóa: đậu cô ve, nguyên tố vi lượng, chỉ tiêu sinh lí, giai đoạn cây con.

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is an industrial plant grown in our country and in the world, not only providing many nutrients for humans but also for both animals and soil improvement very good. Therefore, research into factors that increase drought tolerance as well as productivity for common bean is essential.

Nowadays, research on the effects of micro-nutrients on the yield and tolerance of plant is a topic of concern for many scientists. The study of Nguyen Tan Le (2010) [3] showed that using B, Mn, Cu and Zn apply to the soil, soaking seeds and spraying on the leaves of sesame planted in pots in summer season in Da Nang has increased the tolerance and heat tolerance of sesame. Nguyen Duy Minh (2011) [5] when studying the effect of Mo, Cu, Mn showed that Mo, Cu, Mn affects the physiological indexes at the stage of

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germination and promotes photosynthetic pigmentation, accumulation of organic matter. Nguyen Dinh Thi *et al.* (2008) [6] studied the effects of B, Mo, Zn on physiological indexes and peanut productivity in Thua Thien Hue province: treatment Zn, Mo separately and in combination have the effect of increasing the size of the photosynthetic apparatus (mass leaf area, leaf area) and the concentration of chlorophyll a, chlorophyll b. Tran Khanh Van *et al.* (2014) [7] studied the effect of Mo on the drought tolerance of some soybean cultivars at the seedling stage, indicating that under drought conditions the seedlings of soybean Mo increased total chlorophyll content, increased proline content, water content and root length compared with control, Mo confirmed positive effects on the tolerance mechanism of soybean. These results show that micro-nutrients affect the drought tolerance of some plant, especially the legumes, which can significantly increase crop yields.

In the seedling stage, micro-nutrients affect growth and development, especially some physiological indexes of plants. Therefore, we have studied the effects of Mo, Cu and Mn on some physiological indexes of common bean variety NHPO4 at the seedling stage as a basis for the development of growth, tolerance of plant.

2. Materials and methods

2.1. Time and location of research

2.1.1. Research time

The experiment was conducted from march to may 2017.

2.1.2. Research location

The experiment was arranged at the Department of Botany, Faculty of Natural Sciences, Hong Duc University.

Physiological indexes were analyzed at the Department of Botany, Faculty of Natural Sciences, Hong Duc University.

2.2. Research materials

Common bean variety NHPO4 is produced by the Research Center for Bean Development, Vietnam Academy of Agriculture.

Chemicals: $(NH_4)_2Mo_7O_2.4H_2O$, $CuSO_4.5H_2O$, $MnSO_4.5H_2O$

2.3. Research methods

2.3.1. Method of growing plants in nurseries

Use pots of 20 x 40cm, chisel 6-7 holes around and bottom pot with hole diameter of 0,5cm. Each pot contains 3kg of sand + 0,5kg of NPK + 2kg of manure + 4kg of soil mixed with the purpose of providing nutrients for the bean plant to develop and control the water supply to the plant. The pots were planted on a rain cover ground in the experimental area under natural conditions, with uniform light from all directions with four treatments [5], [6], [7]:

+ Formula I (F I): not add micro-nutrients (control experiment).

+ Formula II (F II): additional solution $(NH_4)_6Mo_7O_{24}.4H_2O$ 0,03%.

+ Formula III (F III): additional solution $CuSO_4.5H_2O$ 0,03%.

+ Formula IV (F IV): addition of solution $MnSO_4.5H_2O$ 0,03%.

* Experimental layout: The experiment was arranged in 4 formulas, each with 3 pots, 30 plants per pot. The pots are planted and maintained in accordance with the process. Daily spray on the leaves in the form of fog spray solution according to the formula.

2.3.2. Sample collection method

Each sample was sampled in 10 plants and sampled in the morning using a uniformly distributed sampling method and taken on the same leaf layer. Finished samples were placed in plastic bags to minimize dehydration, put samples into the laboratory and conduct experiments.

2.3.3. Methods of analyzing some physiological indexes

** Determine the germination rate*

The germination percentage is the percentage of seed germination on the total number of seeds tested. The germination percentage is calculated according to the formula:

$$X\% = \frac{A}{B} \times 100$$

Therein: X is the rate of seed germination; A: total seed germination; B: total is the number of seeds to be planted.

** Determine water holding capacity of leaves*

The leaves of each formula are taken from the same layer (leaf 3,4 layer from the top), each formula takes 5 leaves, repeat 3 times. After the leaves are removed, the leaves are put into a plastic bag to minimize dehydration. The leaves are then transferred to the laboratory for fresh weight (B). Allow the leaves to evaporate under laboratory conditions for a period of 3 hours, and bring the leaves back to the fresh weight after wilting (b). Place the weighed leaves in the oven at 105^{o}C until constant weight, weighing the dried leaves of V [4]. Water holding capacity of leaves (a) is calculated according to the formula:

$$a\% = \frac{B - b}{B - V} \times 100$$

Therein: a: water holding capacity of leaves (% of water loss/total water); B: initial fresh leaf volume (g); b: fresh leaf volume after wilting 3 hours (g); V: dry weight of leaves after drying (g).

** Determination of water content in leaves*

The leaves of each formula were collected in a plastic bag to minimize dehydration, leaving the leaves to the laboratory for rapid fresh weight (B). Place the weighed leaves in the oven at 105^{o}C until constant weight, then weigh the dry weight of the plant after drying (b) [4]. Water content in leaves is calculated according to the formula:

$$A\% = \frac{B-b}{B} \times 100$$

Therein: A: Water content in leaves; B: initial fresh leaf volume (g); b: dry weight of leaves after drying (g).

** Determination of transpiration intensity*

The transpiration intensity was determined by the CI-340 machine manufactured by CID Bio-Science of the United States.

** Determine the total chlorophyll content in leaves*

The total chlorophyll content was determined by the method of Wintermans, De Mots [4]. The total chlorophyll content is calculated by the formula: $A = \frac{C.V}{P.1000}$

Therein: C: Chlorophyll concentration in extract; C_a (mg/l) = 12,7.E_{663} - 2,69.E_{645}; C_b (mg/l) = 22,9.E_{645} - 4,68.$E_{663;}$ $C_{(a+b)}$(mg/l) = 8,02.E_{662} + 20,2.E_{645}; V: volume of extract; P: sample volume; A: Chlorophyll content in fresh sample (mg/g).

2.3.4. Data processing methods

The data was processed using IRRISTAT 5.0 statistical software.

3. Results and discussion

To study the effect of Mo, Cu and Mn on some physiological indexes of NHPO4 variety at seedling stage, we planted NHPO4 variety on experimental pots according to formulas I, II, III, IV. The results of growth and development in the experimental conditions are shown in Figure 1.

F I (H_2O) F II (Mo) F III (Cu) F IV (Mn)

(A)

F I (H_2O) F II (Mo) F III (Cu) F IV (Mn)

(B)

F I (H_2O) F II (Mo) F III (Cu) F IV (Mn)

(C)

Figure 1. *NHPO4 variety after 15 days (A), 20 days (B), 25 days (C)*

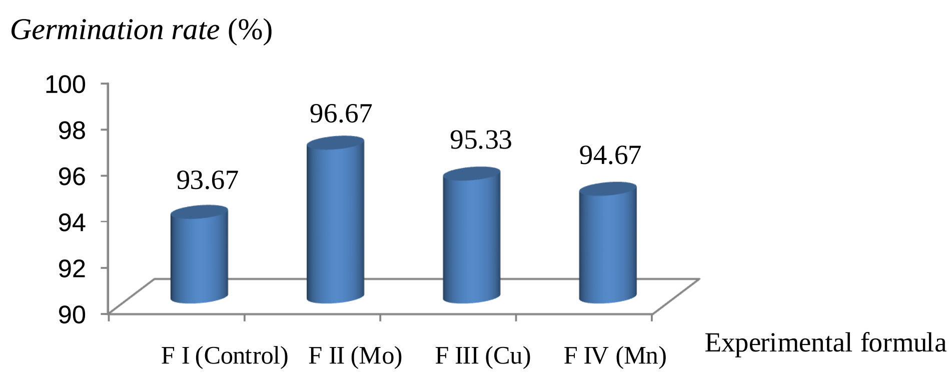
At the stage of the growth and development, we have analyzed the following physiological indexes:

3.1. Germination rate of seeds

Germination is an important physiological process in the growth and development cycle of plants in general and beans in particular which forms the basis of a new body. Seed germination is directly related to the growth of common bean. Research on the effect of micro-nutrients on seed germination has significant implications for improving plant productivity and tolerance. The results are shown in Figure 1.

Figure 1 shows that the germination rate of common bean variety NHPO4 in the formulas was different and showed significant difference. In F I, the lowest germination rate was 93,67%, followed by forrmule Mn with 94,67%, formula applied Cu reached 95,33%, the highest was in formula with Mo germination rate reached 96,67% higher than control 3%.

Chart 1. *Germination rate of NHPO4 variety*



Results of the germination test showed that in the formulas supplemented with micro-nutrients Mo, Cu and Mn had higher germination rates than controls, indicating that micro-nutrients were affected to some biochemical changes during seed germination, enhances metabolic activity within the seed and stimulates seed germination [1], in which

Mo has the most pronounced and positive influence on germination ability of bean seeds.

This result is consistent with that of Nguyen Duy Minh (2011) [5] that Mo and the micro-nutrients for germination were higher than control. Thus, the addition of some micro-nutrients to common beans will increase the germination ability of the seed, which forms the basis for good growth, increasing the ability of drought as well as productivity of the plant.

3.2. Effect of Mo to water contents in leaves

In order for the living organisms of the plant to function normally, the cells need to be watered and reach a water balance, which is reflected by the water content in the leaves. Water content in the leaves have an important role in plant metabolism, especially in relation to transpiration and photosynthesis, thereby affecting productivity and tolerance of plant. Studying water content in leaves at seedling stage is the basis for assessing drought tolerance and yield. The results are shown in Table 2 and Figure 2.

Table 2. *Water content in leaves of NHPO4 variety (%)*

Experimental formula	After 15 days	After 20 days	After 25 days
F I (Control)	$91{,}02^{c} \pm 0{,}02$	$89{,}23^{b} \pm 0{,}02$	$88{,}02^{c} \pm 0{,}03$
F II (Mo)	$93{,}31^{a} \pm 0{,}01$	$90{,}69^{a} \pm 0{,}03$	$90{,}22^{a} \pm 0{,}02$
F III (Cu)	$91{,}06^{c} \pm 0{,}02$	$89{,}36^{b} \pm 0{,}02$	$89{,}03^{b} \pm 0{,}04$
F IV(Mn)	$92{,}09^{b} \pm 0{,}01$	$89{,}58^{b} \pm 0{,}02$	$89{,}22^{b} \pm 0{,}02$

Note: in the same column of data, the values bearing the same letter represent no significant difference, the values bearing different letters reflect the difference in significance level $\alpha = 0{,}05$.

Chart 2. *Water content in leaves of NHPO4 variety*

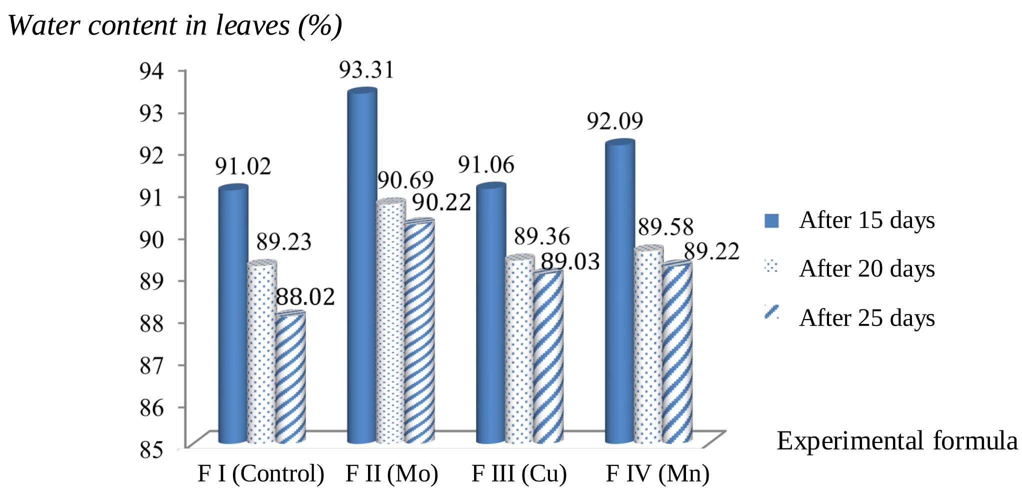


Table 2 and Figure 2 show that the water content of the leaves in all formulas decreased after sowing for 15 to 25 days. After 15 days, in F I, NHPO4 variety had the lowest water content reached 91,02%. Then, F III reached 91,06%, F IV reached 92,09%, the highest rate was 93,31%. After 20 days, F I had the lowest water content of leaves reached 89,23%, followed by F III reached 89,36%, F IV reached 89,58%, the highest was F II reached 90,69%. After 25 days, F I had the lowest water content of leaves reached 88,02%, followed by F III with 89,03%, F IV with 89,22%, FII with Mo 90%,22%.

The results showed that in the control formula does not add micro-nutrients, the water content in leaves is always at the lowest level. At the same time, in F II applied Mo had the highest water content in leaves at all three stages of the study, and showed a statistically significant difference compared to the other formulas. Followed by F IV applied Mn and finally the F III applied Cu. This is due to the fact that Mo has been added that has affected the energy exchange and to the maintenance of high concentrations of protit, nucleic acids, etc., thus affecting hydrophilicity and water holding capacity. Mn is involved in photochemical phosphorylation and hydrolysis of water, and Cu is present in protein - enzymes that increase water holding capacity of plants. Under Mo's influence, glacial linkage increases, leading to increased osmotic water content and reduced osmotic pressure of translation in cells, which increases the viscosity of the protoplasm in cell, thus increasing the tolerance to drought. This result is consistent with the results of Tran Khanh Van *et al.* (2014) on the effect of Mo on the water content of some soybean varieties at the seedling stage.

Thus, the addition of some micro-nutrients (Mo, Mn, Cu) to the common bean at the seedling stage has a positive effect on the water content in leaves, which increases the tolerance of the plant, which is the basis increase the yield of common bean.

3.3. The effect of Mo on water holding capacity of leaves

Water holding capacity is one of the indexes of water balance in plants, which is indicative of holding capacity of hydrophilic cytoplasm against dehydration [2]. Results of water holding of leaves are presented in Table 3 and Figure 3.

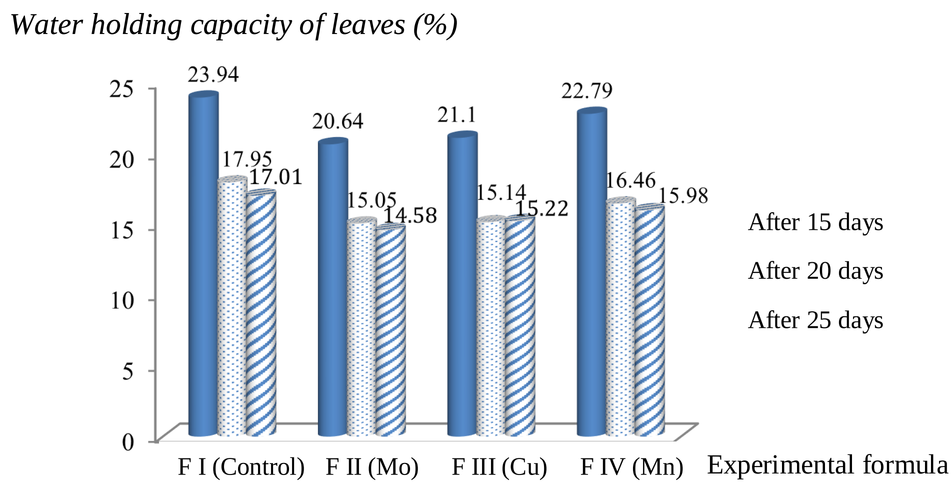
Table 3. *Water holding capacity of NHPO4 variety (% of water lost/total water content)*

Experimental formula	After 15 days	After 20 days	After 25 days
F I (Control)	$23,94^{a} \pm 0,02$	$17,95^{a} \pm 0,03$	$17,01^{a} \pm 0,02$
F II (Mo)	$20,64^{d} \pm 0,04$	$15,05^{c} \pm 0,02$	$14,58^{d} \pm 0,01$
F III (Cu)	$21,10^{c} \pm 0,02$	$15,14^{c} \pm 0,03$	$15,22^{c} \pm 0,01$
F IV (Mn)	$22,79^{b} \pm 0,03$	$16,46^{b} \pm 0,02$	$15,98^{b} \pm 0,02$

Note: in the same column of data, the values bearing the same letter represent no significant difference, the values bearing different letters reflect the difference in significance level $\alpha = 0,05$.

In the formulas with less water loss/total water, the water holding capacity of the leaves as possible. Table 3 and Figure 3 show that the water holding capacity of the leaves increased from 15 days to 25 days. This is fit with the growth of the plant, and the water holding capacity increases as the tissues in the leaves grow older. After 15 days seeding in F I, NHPO4 had the highest water loss/total water reached 23,94%, thus the lowest water holding capacity, followed by F IV reached 22,79%, F III reached 21,10%, in these two formulas NHPO4 has water holding capacity average. Common been in F II have the best water holding capacity because of the lowest water loss/total water eached 20,64%.

Figure 3. *Water holding capacity of NHPO4 variety*



After 20 days in F I, the common bean has the lowest water holding capacity because of the loss of water/total water reached 17,95%, followed by F IV 16,46%, F III reached 15,14%, the highest is in F II reached 15,05%. After 25 days, common bean in F I still have the lowest water holding capacity due to the amount of water lost/total water reached 17,01%, followed by the common been in F IV reached 15,98% and F III applied Cu reached 15,22%. The common been in F II applied Mo have % of water loss/total water reached 14,58% so it has the highest water holding capacity.

It can be seen, at all stages of research, common beans in formula I have the lowest water holding capacity showing statistical difference. Meanwhile, the formulas applied Mo, Mn and Cu have water holding capacity the better, which is most evident in F II applied Mo, then F III applied Cu and F IV applied Mn. Thus, supplementation micro-nutrients at the stage of seedlings for common beans has the effect of increasing the water holding of leaves, ensuring water balance in plant is the premise of increasing the tolerance to drought and common beans yield.

3.2.3. Effect of Mo on transpiration intensity of leaves

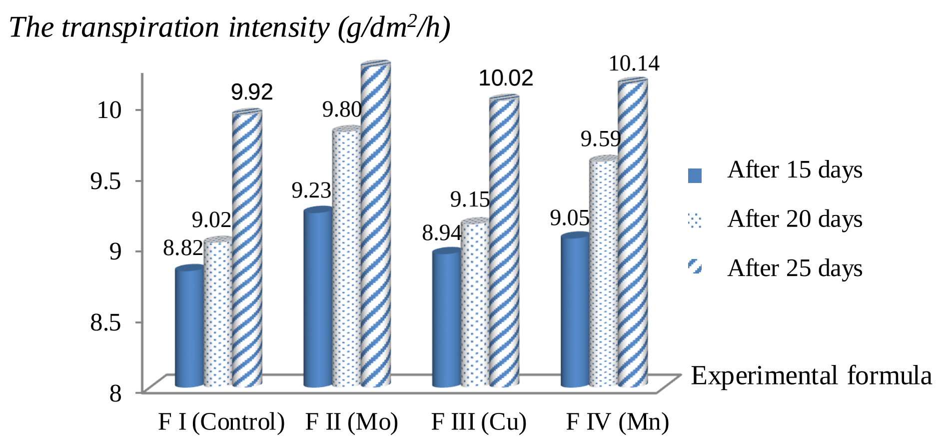
Water evaporation of leaves creates the upper motive of water absorption and reduces the leaf temperature, which helps the leaves to be less heated by sunlight, especially when the transpiration takes place, stoma open will facilitate CO_2 into the interior and O_2 exits to help the plant's physiological processes [2]. This is the basis for increasing biomass, increasing crop yields and tolerance to plants. Strong or weak transpiration of the plants is shown by the transpiration intensity. The results of the study on the transpiration intensity are shown in Table 4 and Figure 4

Table 4. *The transpiration intensity of NHPO4 variety (g/dm²/h)*

Experimental formula	After 15 days	After 20 days	After 25 days
F I (Control)	$8,82^{c} \pm 0,03$	$9,02^{b} \pm 0,02$	$9,92^{b} \pm 0,02$
F II (Mo)	$9,23^{a} \pm 0,04$	$9,80^{a} \pm 0,02$	$10,26^{a} \pm 0,01$
F III (Cu)	$8,94^{b} \pm 0,01$	$9,15^{b} \pm 0,03$	$10,02^{b} \pm 0,02$
F IV (Mn)	$9,05^{b} \pm 0,02$	$9,59^{a} \pm 0,04$	$10,14^{ab} \pm 0,02$

Note: in the same column of data, the values bearing the same letter represent no significant difference, the values bearing different letters reflect the difference in significance level $\alpha = 0,05$.

Figure 4. *The transpiration intensity of NHPO4 variety (g/dm²/h)*



Data table 4 and Figure 4 show that the transpiration intensity of NHPO4 variety increased with different stages of development and different in experimental formulas. After 15 days, NHPO4 variety had the lowest transpiration intensity reached 8,82 $g/dm^2/h$, followed by F III of 8,94 g/dm^2/ h, F IV 9,05 $g/dm^2/h$, the highest is F II reaching 9,23 $g/dm^2/h$.

After 20 days of sowing, in F I the lowest leaf transpiration intensity was 9,02 g/dm^2/h, followed by F III at 9,15 g/dm^2/h and F IV at 9,59 g/dm^2/h, the highest is still F II reached 9,80 g/dm^2/h. After 25 days, F I had the lowest transpiration intensity reached 9,92 g/dm^2/h, followed by F III reached 10,02 g/dm^2/h and F IV reached 10,14 g/dm^2/h, the highest is still in F II applied Mo reached transpiration intensity of 10,26 g/dm^2/h.

Results show that the Mo has the highest effect on the transpiration intensity in all the formulas, indicating significant differences, then Mn and Cu, which proves Mo, Cu and Mn micro-nutrients have been implicated in physiological activities in the plant, maintaining the water balance in the plant layers, Maintaining the properties of the cytoplasmic substance in the cell facilitating metabolism of the plant works better.

3.2.4. Chlorophyll content in leaves

Chlorophyll is the major photosynthetic pigment of plants, which is an important role in photosynthesis. The density of chlorophyll in the leaves is an important role in assessing the photosynthesis of plants, which in affects crop yields. The results of study about chlorophyll content in leaves are presented in Table 5 and Figure 5.

Table 5. *Chlorophyll content of NHPO4 variety (mg/g)*

Experimental formula	After 15 days	After 20 days	After 25 days
F I (Control)	$0{,}59^{bc} \pm 0{,}01$	$0{,}68^{c} \pm 0{,}02$	$0{,}83^{c} \pm 0{,}02$
F II (Mo)	$0{,}71^{a} \pm 0{,}01$	$0{,}85^{a} \pm 0{,}01$	$0{,}96^{a} \pm 0{,}02$
F III (Cu)	$0{,}62^{b} \pm 0{,}02$	$0{,}72^{c} \pm 0{,}02$	$0{,}84^{c} \pm 0{,}01$
F IV (Mn)	$0{,}65^{b} \pm 0{,}01$	$0{,}77^{b} \pm 0{,}01$	$0{,}89^{b} \pm 0{,}02$

Note: in the same column of data, the values bearing the same letter represent no significant difference, the values bearing different letters reflect the difference in significance level $\alpha = 0{,}05$.

Table 5 and Figure 5 show that the chlorophyll concent in the leaves of NHPO4 variety increased with different stages of development and different in experimental formulas. After 15 days, in F I, NHPO4 had the lowest chlorophyll content in leaves reached 0,59 mg/g, followed by F III reached 0,62 mg/g, F IV reached 0,65 mg/g, the highest was F II reached 0,71 mg/g. After 20 days, in F I, NHPO4 variety had the lowest chlorophyll content in leaves reached 0,68 mg/g, followed by F III reached 0,72 mg/g, F IV reached 0,77 mg/g, the highest is F II reached 0,98 mg/g. After 25 days, in F I, NHP04 variety has the lowest chlorophyll content in leaves reached 0,83 mg/g, followed by F III reached 0,84 mg/g, F IV reached 0,89 mg/g, the highest was F II reached 0,96 mg/g.

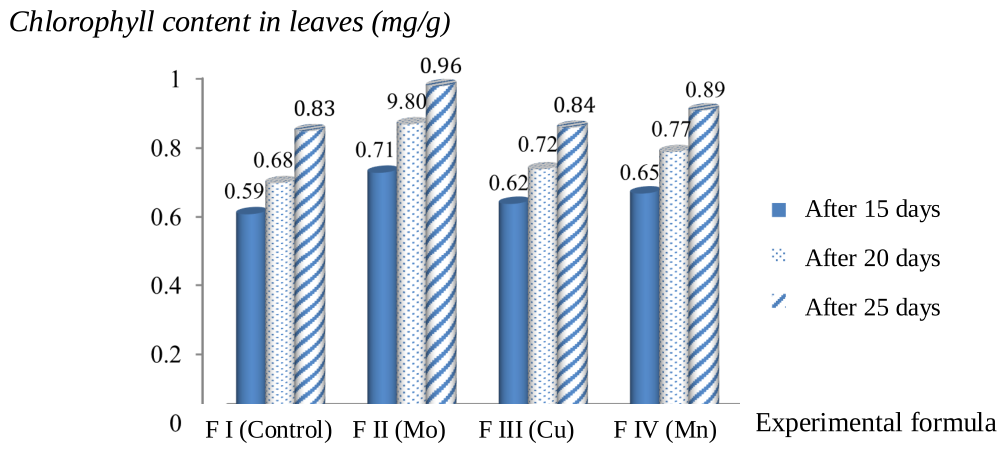


Figure 5. *Chlorophyll content in leaves of NHPO4 variety*

In formulas Cu, Mn or Mo, the content of chlorophyll is higher than that of non-supplemented formulas. In that in F II applied Mo, the total chlorophyll content in leaves was highest at all stages, the values were significantly different from those of the other, followed by formulas applied Mn and Cu.

The results of this study are consistent with research by Nguyen Duy Minh (2011) [5] that Mo, Cu, Mn have the effect of promoting photosynthetic pigment, in accordance with research by Tran Khanh Van *et al.* [7] that Mo increases the total chlorophyll content in leaves because Mo increases the binding ability of chlorophyll to the protein-lipoic complexes, reducing the chlorophyll breakdown, this facilitates the normal photosynthesis of plants.

4. Conclusion

Mo, Cu and Mn micro-nutrients directly affect some physiological indexes (germination rate, water holding capacity, water content in leaves, transpiration intensity, chlorophyll content of common bean variety NHPO4 at the seedling stage, in formulas applied Mo, Cu, Mn, NHPO4 variety showed some physiological indexes better than control.

Of the three elements Mo, Cu, Mn, Mo was the most significant influence on some physiological indexes. Therefore, in general cultivation and cultivation of common beans, it is necessary to supplement micro-nutrients, especially Mo for plants in the early stages to promote the growth of the plant, creating a premise for the plants, create conditions for high yield, tolerant to adverse conditions of environment.

Conflict of Interest: *Authors have no conflict of interest to declare.*

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