

**Vladan POPOVIĆ, Tatjana ĆIRKOVIĆ-MITROVIĆ,
Aleksandar LUČIĆ and Ljubinko RAKONJAC¹**

**EFFECT OF FERTILIZERS ON CONCENTRATION OF
PHOTOSYNTHETIC PIGMENTS IN LEAVES OF ONE-YEAR-OLD
SEEDLINGS OF WALNUT (*Juglans regia* L.)**

SUMMARY

The effect of three different fertilizers on concentration of photosynthetic pigments in leaves of one-year-old seedlings of walnut (*Juglans regia* L.) has been studied in the nursery conditions. Three types of pigments have been examined: chlorophyll a, chlorophyll b and carotenoids as well as the total concentration of chlorophyll a and b. The trial was set up in random block system with three types of fertilizers and a control in three replications in the nursery of Institute of forestry in Belgrade in 2013. The leaf sampling was carried out in the middle of the growing season. The highest concentration of photosynthetic pigments was found in the leaves of seedlings treated with the preparation Bactofil B 10 and the lowest in the seedlings treated with mineral fertilizer NPK 15:15:15. The highest mean value had chlorophyll b (0,486 mg/g) in treatment Bactofil B 10 and the lowest mean value had carotenoids (0,165 mg/g) in treatment NPK 15:15:15. Research showed that the concentration of photosynthetic pigments in leaves of walnut seedlings varied depending on the fertilizer that was applied. Based on the obtained results it can be concluded that the proper fertilizer can increase the concentration of photosynthetic pigments in leaves and therefore the intensity of photosynthesis which contributes to increasing the biomass production.

Key words: Walnut, fertilizer, photosynthetic pigments, leaves.

INTRODUCTION

Importance of photosynthetic pigments is reflected in the absorption of light necessary for the process of photosynthesis in plants. Chlorophylls are the primary photosynthetic pigments. They reflect green part of the spectrum of visible light, while carotenoids reflect yellow, orange or red part of the spectrum. The ability to absorb certain wavelengths of light is more important for the process of photosynthesis than the ability of reflection of the part of the light.

From the aspect of plant nutrition the use of various nutrition preparations can significantly influence the concentration of pigments and thus on photosynthesis as a whole (Janmohammadi et al., 2012). This paper presents

¹ Vladan Popović* (corresponding author: vladanpop79@gmail.com), Tatjana Ćirković-Mitrović, Aleksandar Lučić, Ljubinko Rakonjac, Institute of Forestry, 11030 Belgrade, SERBIA
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study of the effect of various nutrition preparations on pigments in leaves of walnut (*Juglans regia* L.) one-year-old seedlings and their mutual correlation was determined.

MATERIAL AND METHODS

The experiment was set up in a random block system with three types of fertilizers and a control in three replications in the nursery of Institute of forestry in Belgrade in 2013. The nursery is located at 20° 27' 44" east longitude and 44° 49' 14" north latitude.

For establishment of the trial was used the walnut seed collected in 2012. The walnut tree from which the seed was collected is located in vicinity of city Leskovac in a place called Graovo at 44° 45' 34" north latitude and 22° 06' 05" east longitude, at elevation of 520 m. Due to embryo dormancy the walnut seed was moist stratified from November 2012 until end of March 2013 (at the temperature 3-5 °C). The sowing was performed in rows at a depth of 8 cm in April 2013. The seed was planted in Tref TPS fine brown substrate. Except the control block which was not treated with nutrition preparations, three blocks of trial fields were treated with three types of fertilizers: the controlled release fertilizer Osmocote® Exact Standard 5-6 M, the microbiological preparation Bactofil® B 10 and the complex NPK mineral fertilizer Florin 2, the dosage is recommended by the manufacturers (Čirković-Mitrović, 2014).

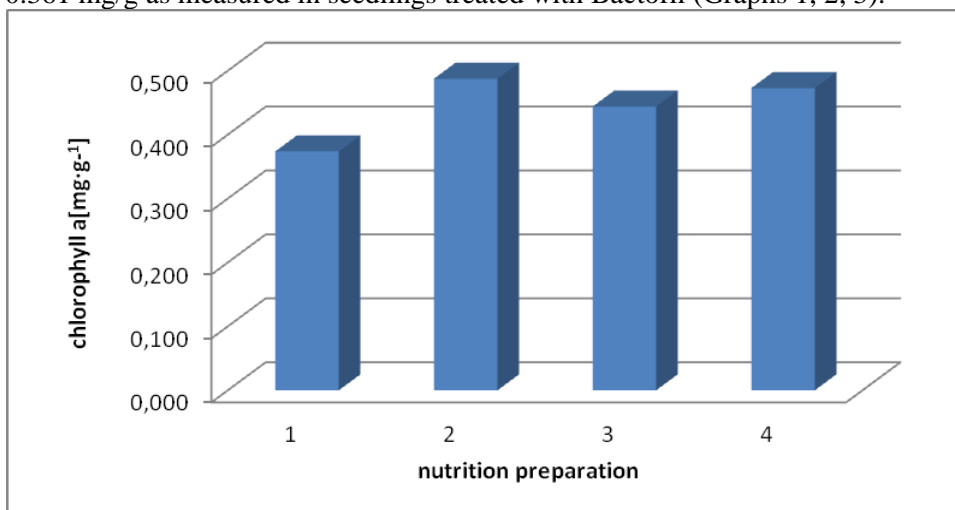
Leaf sampling of walnut seedlings was conducted in mid- growing period. A sample of 1g was homogenized using a mortar and pestle. For better homogenization of the sample 2 g of quartz sand was added in the mortar before mechanical grinding. The paste was for 3 minutes treated with 15 ml of 80% acetone. To this mixture was added 1 mg of MgCO₃ in order to prevent acidification of the solution. The resulting green solution was applied by a small glass rod on a glass filter and using a water spray vacuum pump it was filtered into the vacuum test tube. The resulting filtrate was the pigment extract which is transferred from the test tube to the regular 25 ml vessel and supplemented with 80% acetone to the line. To perform reading in a spectrophotometer the obtained extract has to be diluted. 1 ml of the obtained extract was taken by pipette and into that was added 9 ml of acetone and then it was transferred to the test tube. Thus prepared extract was poured into the cuvette and read on the spectrophotometer, the absorption was at the wavelengths 662, 644 and 440 nm. The formula of Holm and Wetstein was applied to calculate the concentration of the pigment in the extract in mg / dm³.

Preparing and reading on the spectrophotometer as well as calculating was performed using standard methods (Oljača and Srdić, 2005).

RESULTS AND DISCUSSION

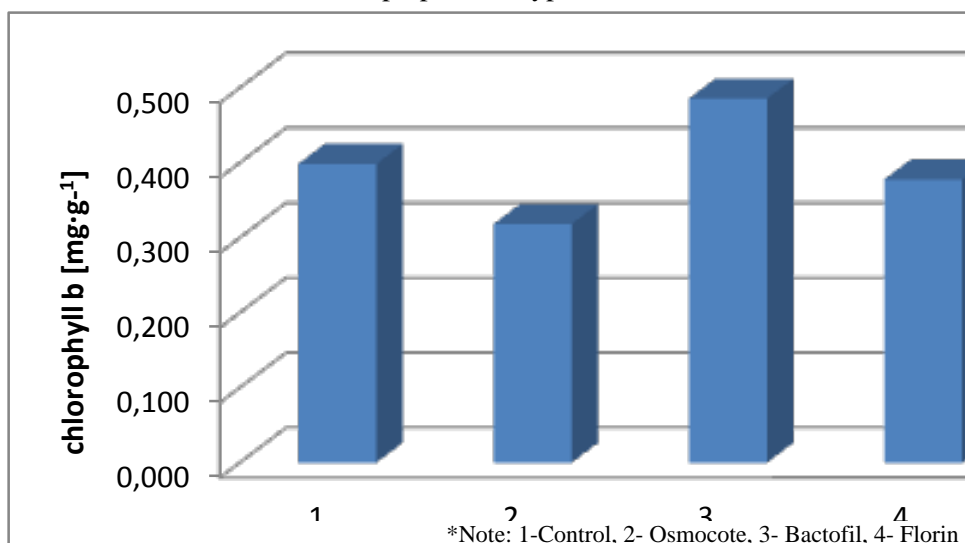
Table 1 presents the concentration of photosynthetic pigments in walnut leaves depending on the applied nutrition preparation. When observing the concentration of chlorophyll a, it increases in seedlings treated with nutrition

preparations compared to non-treated seedlings. The greatest concentration of chlorophyll a in the amount of 0.488 mg/g was measured in the seedlings treated with Osmocote and the lowest in control seedlings in the amount of 0.374 mg/g. The concentration of chlorophyll b was in the range of 0.318 to 0.486 mg/g. The highest concentration was measured in seedlings treated with Bactofil and the lowest in seedlings treated with Osmocote. The concentration of carotenoids was ranging from 0.165 mg/g which was measured in seedlings treated with Florin to 0.361 mg/g as measured in seedlings treated with Bactofil (Graphs 1, 2, 3).



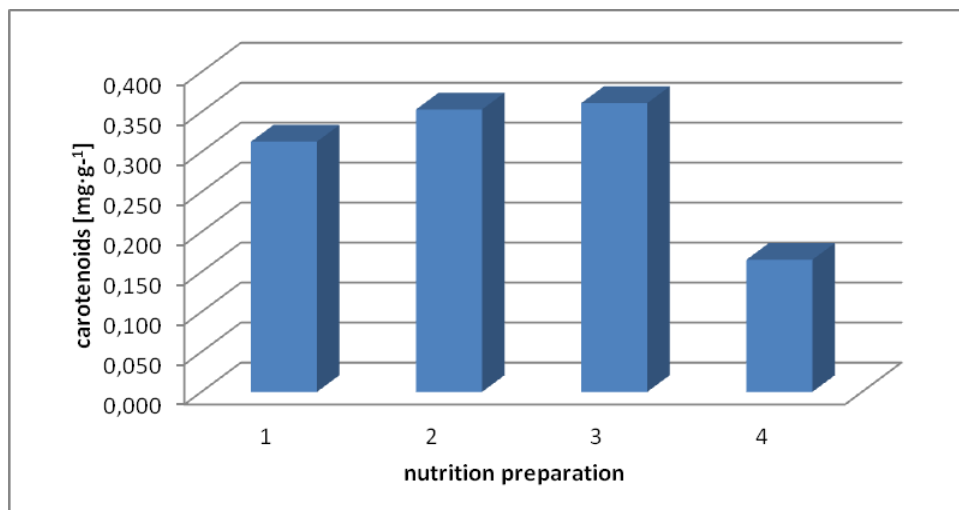
*Note: 1-Control, 2- Osmocote, 3- Bactofil, 4- Florin

Graph 1. Concentration of chlorophyll a in walnut leaves depending on nutrition preparation type



*Note: 1-Control, 2- Osmocote, 3- Bactofil, 4- Florin

Graph 2. Concentration of chlorophyll b in walnut leaves depending on nutrition preparation type



*Note: 1-Control, 2- Osmocote, 3- Bactofil, 4- Florin

Graph 3. Concentration of carotenoids in walnut leaves depending on nutrition preparation type

A foliar concentration of the main photosynthetic pigments chlorophyll a and b is considered to be a bio-indicator of the total primary production of biomass (Gitelson and Merzlyak, 1994). The results showed that the addition of the nutrition preparations in the substrate in the first year of walnut seedlings growth positively affected the concentration of photosynthetic pigments in the leaves of these plants. The research results of some authors also showed that the fertilization has a positive effect on concentration of photosynthetic pigments and quality of plants (Bravdo et al., 1993, Tojnko et al., 2001, Nellsen et al., 2003, Ćirić et al., 2005). The greatest concentration of pigments was in walnut seedlings that were treated with the microbiological preparation Bactofil. It also positively affected on development and morphometric characteristics of walnut seedlings (Ćirković-Mitrović, 2014a). The positive effect of microbiological preparations on the quality, morphometric characteristics and yield of plants in this region was also determined in other researches (Kristek et al., 2010; Dolijanović et al., 2014; Ćirković-Mitrović, 2014).

CONCLUSIONS

The concentration of photosynthetic pigments in walnut leaves increases when the seedlings are treated with nutrition preparations. The best results showed the preparation Bactofil because the concentration of all observed pigments increased in relation to other nutrition preparations and certainly in relation to the non-treated seedlings. Bactofil as a microbiological preparation is highly desirable in nursery production due to its basic characteristics, because except the positive effect on plant development it improves the characteristics of the substrate as well and it is not harmful to the environment.

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