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Photosynthetic abilities and essential oil content of hydroponic and soil *Thuja occidentalis*

Anahit Hakobjanyan, Astghik Karapetyan, Aristakes Ghahramanyan, Anna Yeghiazaryan, Armen Gasparyan, Khachatur Mayrapetyan

G. S. Davtyan Institute of Hydroponics Problems, NAS RA, Yerevan, Armenia

Corresponding Author: Anahit A. Hakobjanyan, G. S. Davtyan Institute of Hydroponics Problems, NAS RA, Noragyugh 108, Yerevan, 0082, Armenia

Submission Date: September 4th, 2024; **Acceptance Date:** October 16th, 2024**; Publication Date:** October 23rd, 2024

Please cite this article as: Hakobjanyan A., Karapetyan A., Ghahramanyan A., Yeghiazaryan A., Gasparyan A., Mayrapetyan K. Photosynthetic abilities and essential oil content of hydroponic and soil Thuja occidentalis. Bioactive Compounds in Health and Disease 2024; 7(10): 536-543. DOI: https://www.doi.org/10.31989/bchd.v7i10.1457

ABSTRACT

Background: *Thuja occidentalis* is used in folk medicine to treat several diseases, like bronchial catarrh, and rheumatism. It exhibits antimicrobial properties, and many people today incorporate Thuja occidentalis leaves into herbal tea blends for their healing potential. Brewing tea involves extracting chlorophylls, carotenoids, and essential oils from the plant, which may enhance the tea's therapeutic benefits.

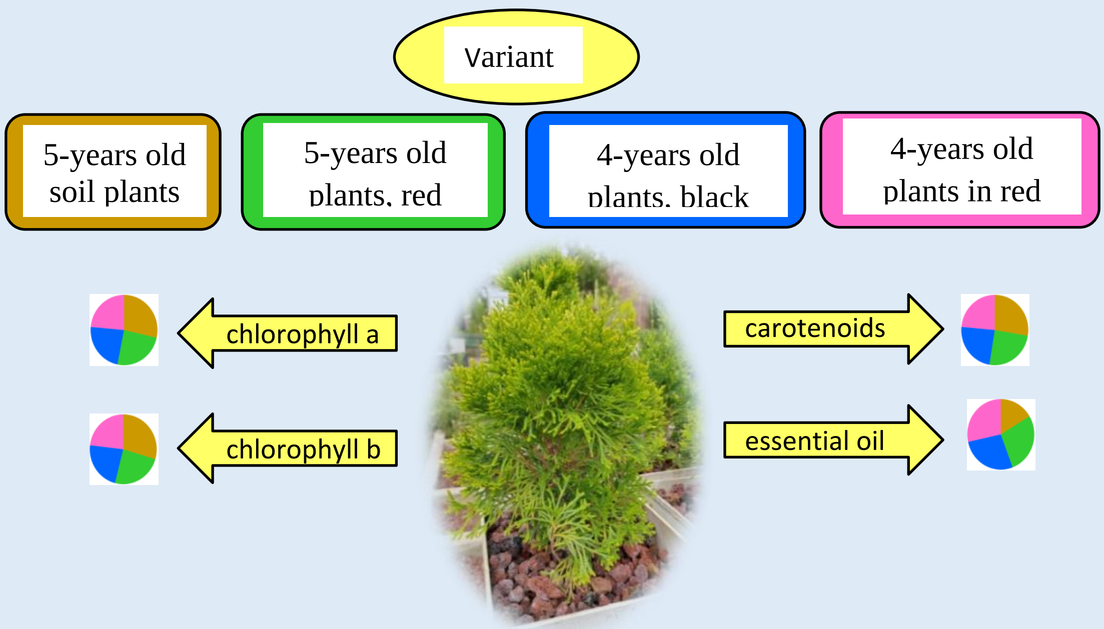
Objective: The aim of the study was to estimate the content of chlorophyll a and b, carotenoids, and essential oils in the leaves of *Thuja occidentalis* grown in Ararat Valley under hydroponic and soil conditions and compare the herbal properties of this plant depending on the growth conditions.

Methods: Plants of *Thuja occidentalis* were grown under soil and hydroponic conditions. The hydroponic substrates used red and black slags. Hydroponic plants were nourished with the nutrition solution offered by G. Davtyan. The content of chlorophyll a and b, and carotenoids were estimated spectrophotometrically in an alcohol solution of leaves. The essential oil of plant leaves was extracted by hydrodistillation.

Results: In our study, the content of chlorophyll a and b and carotenoids increased in all studied plants from May to July. The highest content of them was recorded in soil plants (chlorophyll a – 7.2-8.8 µg/ml, chlorophyll b – 2.5-3.2 µg/ml and carotenoids – 1.8-2.2 µg/ml). Plants grown in red slag showed slightly higher levels of these components compared with plants grown in black slag, especially in May. The content of essential oil was higher in hydroponic plants (0.5-0.52%), compared with the soil plants (0.3%).

Conclusion: Our study showed that the content of chlorophyll a and b and carotenoids in leaves of *Thuja occidentalis* changes depending on the studied month and soil plants are inferior to the hydroponic plants in essential oil content. All this supports the idea that the healing abilities and properties of *Thuja occidentalis* leave depend on the harvesting time and growth condition.

Keywords: chlorophyll a and b, carotenoids, essential oil, Ararat Valley, hydroponics



INTRODUCTION

Thuja occidentalis is one of the high-rateable decorative plants used in city greenings and yard decorations. It is in great demand among gardeners. But it is valuable not only because of its use in greening. It is used in homeopathy to treat rheumatoid arthritis and warts [1-2]. In folk medicine, *Thuja occidentalis* is used to treat various diseases, such as bronchial catarrh, cystitis, psoriasis, amenorrhea, and rheumatism. Plant leaves are effective against both gram-positive and gram-negative bacteria, with carotenoids contributing to this activity [3]. Nowadays many people try to use leaves of *Thuja occidentalis* in herbal tea compositions, considering its healing abilities. In markets, there are many offers of tea from *Thuja occidentalis*, but there is not any scientific study directly done on this tea. Making tea means the extraction of chlorophylls, carotenoids, and essential oils of tea plants into tea solution. These extracted components may affect the tea's healing abilities.

Chlorophylls are the main components of plant photosynthesis. Their amounts are correlated with plant growth [4]. Chlorophylls may be good antioxidants, antimutagens, and anticarcinogens. Their use in diet may protect human organisms from obesity, diabetes, cardiovascular diseases, and atherosclerosis [5]. Chlorophyll derivatives help in detoxifying the organism, eliminating heavy metals and toxins and promoting the growth of beneficial gut microbiota [6].

Carotenoids possess antioxidant activity and have anti-inflammatory effects. They are important for the eye, bone, cardiovascular, skin health, and immune system. One method of extracting carotenoids is hydrothermal, which uses water at high temperatures and pressure to extract carotenoids [7]. This is like the process of making tea. Carotenoids have potential prebiotic effects [8].

Thuja's essential oil may inhibit the growth of gram-positive and gram-negative bacteria [9].

Hydroponic plant growth promotes water conservation, which is crucial given today's ecological issues related to water scarcity. It may also help to regulate the content of needed bioactive compounds in plants.

Here we evaluated the content of chlorophylls, carotenoids and essential oil in leaves of *Thuja occidentalis* grown in soil and hydroponics to see if the two growth methods are equivalent or not.

MATERIAL AND METHODS

Plants of *Thuja occidentalis* were grown in soil and hydroponic conditions in the Ararat Valley on the territory of the Davtyan Institute of Hydroponics Problems. Soil plants were 5 years old (n=5). They were nourished by artesian water once a week. There were 3 variants of hydroponic plants: 5-year-old plants grown in black volcanic slag (n=7), 4-year-old plants grown in black volcanic slag (n=10), and 4-years old plants grown in red volcanic slag (n=10). One of the main differences between red and black slags is their different abilities to absorb environmental heat and light that may influence plant growth. Hydroponic plants were nourished with the nutrition solution offered by G. Davtyan [10].

The content of chlorophyll a and b and carotenoids were estimated spectrophotometrically in an alcohol solution of leaves [11]. 0.5g of leaf tissue was gridded by mortar with 10mL 96% ethanol. The received solution was centrifuged for 5 min at 11000g. 0.5mL of supernatant was taken and diluted with 4.5ml ethanol. The optical density of the final solution was measured at wavelengths 470nm, 649nm, and 664nm. The following formulas were used to calculate the content of chlorophyll a and b, and carotenoids in µg/mL:

$$Chlorophyll_{\mathrm{a}} = 13.36A_{664} - 5.19A_{649}$$

$$Chlorophyll_{\mathrm{b}} = 27.43A_{649} - 8.12A_{664}$$

$$Carotenoids = (1000A_{470} - 2.13Chl_a - 97.63Chl_b)/209$$

Fig. 1. The view of 5-year-old *Thuja occidentalis* (a) in soil and (b) in hydroponics (black slag).

The amount of calcium (Ca) and magnesium (Mg) was determined using the ash of thuja's needles [12], which was derived by burning needles for 8 hours at 450ºC. 0.2 g of ash was solved in 1 mL distilled water and 2 mL HCl (concentrated HCl was diluted with distilled water in a ratio of 1:1). The solution was evaporated in a water bath. 2 mL HCl[1] and 3 mL boiling distilled water were added to the remained residue. The received solution was filtered by ash-free filter. The filter was washed until the filtrated solution (FS) reached 100 mL. 20 mL of FS was added to 30 mL of distilled water. The received solution was colored with 1-2 drops of malachite green alcohol solution to receive a light bluish color. The solution was then discolored with 5M solution of KOH, after which an additional 2 mL of the KOH solution was added, then some dust of murexide to colorize the solution into pink. The solution was titrated by Trilon B (0.1N) until the color was changed into a light pink color. The amount of Ca was calculated:

$$Ca = \frac{2 \times N \times V}{M}$$

where N is the normality of Trilon B, V is the volume (ml) of Trilon B used in the titration, and M is the amount of ash used.

20 mL of FS was mixed with 30 mL of distilled water. The received solution was colored with 1 drop of methyl red alcohol solution to receive a pink color. Then 5M solution of KOH was added until the solution color changed to yellowish, after which 2 mL of the ammonium chloride buffer was added, then some crystals of Eriochrome Black T to colorize the solution into wine color. The solution was titrated by Trilon B (0.1N) until the color was changed to azure color. The amount of Mg was calculated:

$$Mg = \frac{1.2 \times N \times (V_1 - V)}{M}$$

[1] concentrated HCl was diluted with distilled water in a ratio 1:1

where N is the normality of Trilon B, V_1 is the volume (ml) of Trilon B used in the titration, V is the volume (ml) of Trilon B used in the titration of the same sample in the previous test for Ca, M is the amount of ash used.

Essential oil of plant leaves was extracted from the fresh leaves of the plant by hydrodistillation [13].

Statistical analyses were done by GraphPad Prism 5. $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

In our study, the content of chlorophyll a and b and carotenoids increased in all studied plants from May to July. The highest content of them was recorded in soil plants (chlorophyll a – 7.2-8.8 µg/ml, chlorophyll b – 2.5-3.2 µg/ml, and carotenoids – 1.8-2.2 µg/ml) (Fig. 2).

In soil plants, the significant monthly increase of chlorophyll a and b was recorded in July, while for carotenoids it was recorded each month ($p<0.05$). Soil plants have more pigments than hydroponic ones: the difference was higher in May and less in July. In hydroponic setups, plants grown in black slag performed worse than those in red slag, particularly in May. This difference may be attributed to the varying nutrient contents of the two materials. Red-colored slag has iron oxide (giving red color) and some impurities of magnetite and lepidocrite. Black-colored slag consists of crystallized magnetite with a small amount of maghemite [14]. Iron oxide nanoparticles promote the growth and development of plants and enhance their stress tolerance. They support plant nutrition and are good sorbents against hazardous contaminants such as arsenic [15]. The influence of the red slag on the plant height increase is visible in the Fig. 3.

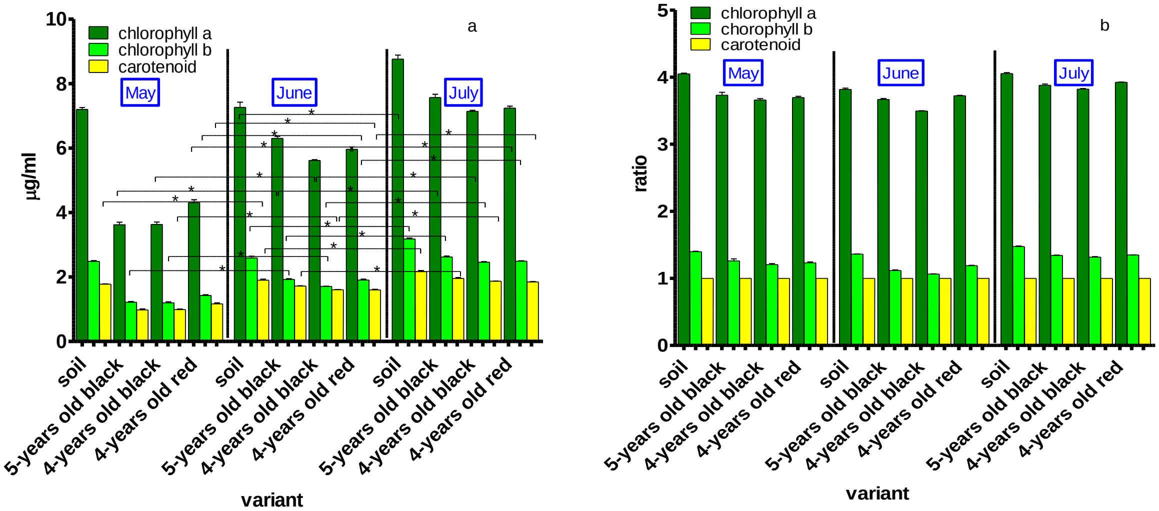


Fig. 2. The content of chlorophyll a and b and carotenoids (a) and chlorophyll a/chlorophyll b/carotenoids ratio in studied variants of *Thuja occidentalis* from May to July (there are significant differences in the same variant in different months): * - $p<0.05$.

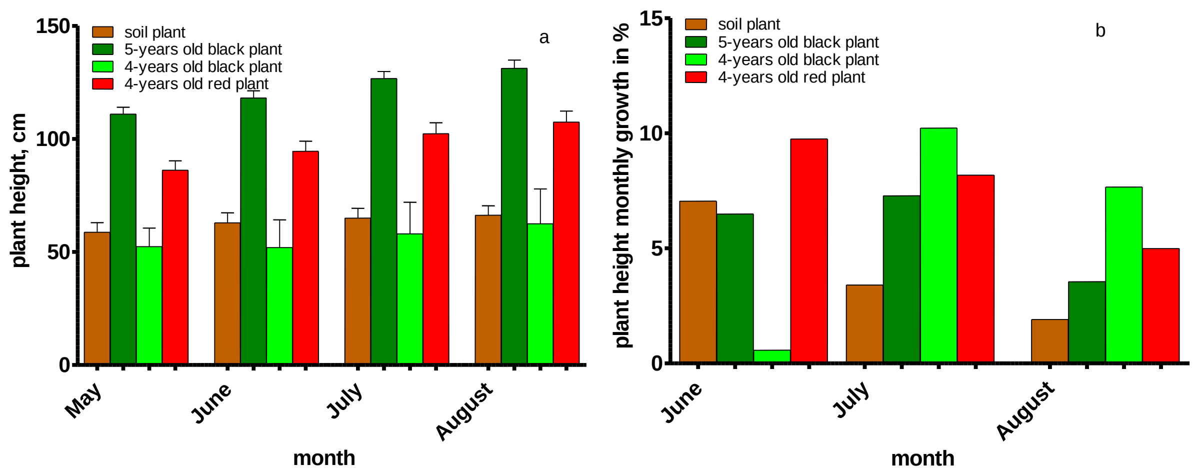


Fig. 3. The height of *Thuja occidentalis* and its monthly growth in % ratio in studied variants from May to July.

It was shown that when soil temperature was higher than the air temperature in the crown, the growth of the apical and side shoots of *Thuja occidentalis* slowed down, the length was reduced, and the number of chlorophylls (a + b) and carotenoids in its needles decreased [16]. The black slag absorbs more heat than red because of the color. This may also influence height and pigment differences between 4-year-old plants grown on these two substrates, especially in May and June. In a study of Kycheryavy [16] the content of chlorophyll a was between 0.38-0.73 mg/g, the content of chlorophyll b was between 0.14-0.35 mg/g and the content of carotenoids was between 0.13-0.27 mg/g. In our study, taking into account that our results are in µg/mL and to change them to mg/g we need to multiply them with 0.2, we have the following results: for soil plants - 1.44-1.75 mg/g chlorophyll a, 0.5-0.64 mg/g chlorophyll b and 0.36-0.43 mg/g carotenoids; for 5 years-old plants - 0.72-1.51 mg/g chlorophyll a, 0.24-0.52 mg/g chlorophyll b and 0.2-0.39 mg/g carotenoids; for 4 years-old plants from black slag - 0.73-1.43 mg/g chlorophyll a, 0.24-0.49 mg/g chlorophyll b and 0.2-0.37 mg/g carotenoids; and for 4 years-old plants from red slag - 0.86-1.45 mg/g chlorophyll a, 0.29-0.5 mg/g chlorophyll b and 0.23-0.37

mg/g carotenoids. Chlorophyll results were tied with the results of Mg (Fig. 4a), which is one of the main particles of the chlorophyll molecule. The highest level of Mg was recorded for soil plants. The level of Ca was higher in plants grown in red slag and the lowest results were recorded for black slag plants.

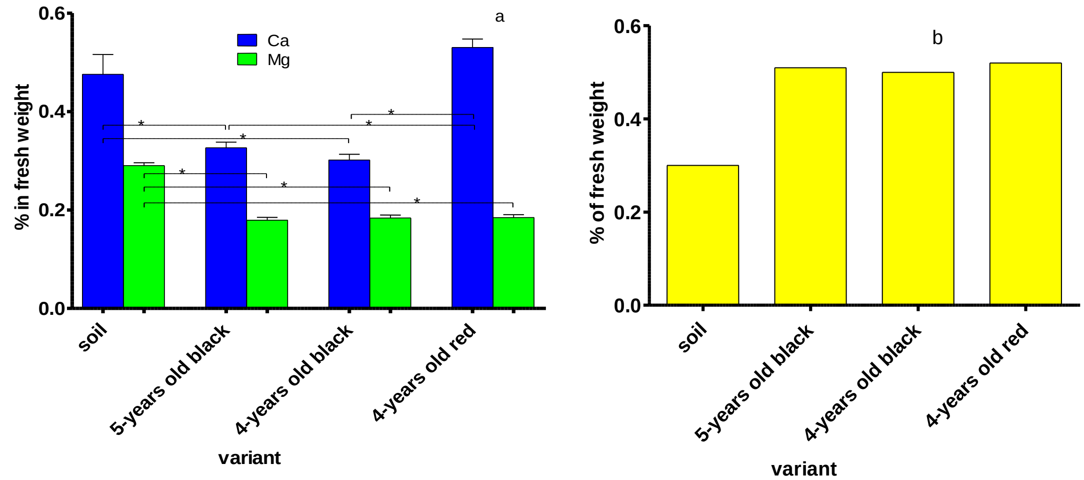


Fig. 4. The content of Ca and Mg (a) and essential oil (b) in studied variants of *Thuja occidentalis*: * - $p<0.05$.

Chlorophyll a has a blue-green color and chlorophyll b is - yellow-green color. At higher temperatures chlorophyll a degrades more quickly than chlorophyll b, which is why herbal teas have a dull yellow-green color [17-18].

Carotenoids in food are part of a healthy human life [19]. They have cytoprotective and photoprotective properties and may protect from obesity and neurodegenerative disorders [20]. Carotenoids protect cells from reactive oxygen species and oxidative damage [21]. It was shown that β-carotene content is higher in the ground tea infusions than in the whole tea infusions [22].

In our study, soil plants were inferior to hydroponic ones in content of essential oil (soil plants - 0.3%, hydroponic plants - 0.5-0.52%). In all variants, the received oil was transparent, not colorized, and had a light sweet odor. It was shown that essential oils of Thuja occidentalis have more antimicrobial activity than alcohol extracts [23]. During brewing herbal tea its strong aroma is conditioned with the presence of plant essential oil [24]. Since the main bioactive component of *Thuja occidentalis* is α-thujone, which is a neurotoxin [25], people should be careful not to overuse Thuja tea.

CONCLUSION: Our study revealed that the levels of chlorophyll a, chlorophyll b, and carotenoids in the leaves of Thuja occidentalis vary by month. While soil-grown plants surpassed hydroponic plants in chlorophyll and carotenoid content, they were found to have lower levels of essential oils. In hydroponics, red slag plants have more chlorophylls and carotenoids than same-aged plants of black slag variant. This supports the idea that the healing abilities and properties of *Thuja occidentalis* leaves depends on the harvesting time and growth condition.

Acknowledgments: The work was supported by the Science Committee of MESCS RA, in the frames of the research project № 21T-4D262.

Abbreviation: FS - filtrated solution.

Competing Interests: The authors have no financial interests or any other conflicts of interest to disclose.

Authors' Contributions: All authors contributed to this review.

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