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Efficacy and selenium enrichment of *Lycium barbarum* in hydroponic and soil conditions in the Ararat valley

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ABSTRACT

Background: It is known that 60-80% of the population affected by various diseases, such as heart attack, stroke, and oncological diseases is associated with selenium (Se) deficiency. Therefore, enriching agricultural crops with Se is considered a current issue.

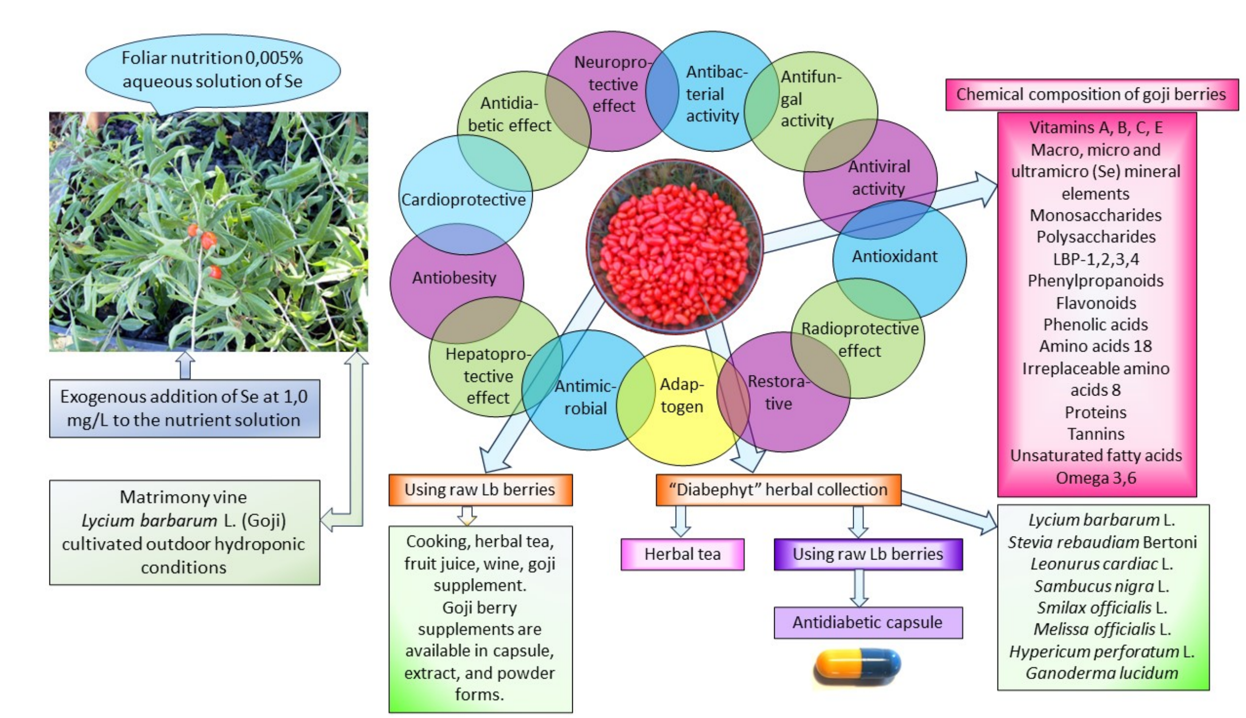
Objective: This study investigates Se enrichment of *Lycium barbarum* (Lb) and its effectiveness in hydroponic and soil conditions in the Ararat Valley. The research focuses on the impact of adding exogenous Se on plant productivity, Se accumulation, and the content of biologically active substances (BAS) in Lb berries and leaves.

Methods: The experiments were conducted from 2021 to 2023 using hydroponic and soil cultivation methods with varying Se concentrations in nutrient solutions and foliar nutrition.

Results: The results demonstrate that Se enrichment significantly increased Se and BAS levels in both leaves and berries of Lb, with hydroponic cultivation exhibiting higher accumulation compared to soil conditions. Foliar feeding with a 0.005% Se solution and exogenous Se addition to nutrient solutions at 1.0 mg/L resulted in notable improvements in Se content and plant productivity. Furthermore, the analyses revealed significant differences between hydroponically and soil-grown Lb, emphasizing the potential of hydroponic cultivation for producing Se-enriched plant material with enhanced BAS content.

Conclusion: Hydroponic cultivation supplemented with Se and foliar nutrition is recommended for obtaining Se-rich Lb plant material, which is suitable for medicinal and functional food purposes. These findings contribute to the development of biotechnologies for Se -enriched plant production and addressing health and nutritional needs.

Key words: Goji, berries, biologically active substances, flavonoids, phenolic acids, tannins, carotenoids



LYCIUM BARBARUM (GOJI) AS A BIOLOGICALLY ACTIVE COMPOUND AND FUNCTIONAL FOOD

INTRODUCTION: Negative human influence on the environment has brought ecological problems like global warming, desertification, loss of biodiversity, environmental pollution, soil erosion, reduction of forest areas, etc. The results of this are degraded ecosystems, damaged biodiversity, and various human diseases, including chronic and infectious. The use of functional foods and therapeutics derived from natural resources may reduce the incidence of chronic and viral diseases and protect from health risks. As a combination of bioactive compounds, functional foods beneficially influence health [1-3].

Lycium barbarum L. (Lb) originates from China and is commonly known as the Tibetan goji berry (Figure 1). It is cultivated in China, Tibet, Mongolia, Japan, Korea, Taiwan, Australia, America, and Northern Africa at altitudes more than 3000 meters above sea level, often near river valleys. Lb is a deciduous shrub, propagated by seeds, cuttings, and root layering. In its habitat, it can withstand cold down to -32°C. It is drought-resistant and undemanding to the soil but is a light-loving plant. LB has been used in food since around 650 AD. Its berries are widely used not only in China, but also in many countries around the world. They are valued for their nutritional and medicinal properties. Traditional Tibetan medicine revered goji berries as a valuable remedy for various illnesses. In eastern countries, Lb berries are considered a source of longevity and vitality. They have gained popularity in Europe due to many health benefits [4-25].

As a food supplement, LB berries regulate the level of minerals such as calcium and potassium, minimize oxidative stress, boost immunity, normalize blood pressure, enhance cognitive function, and remove toxins from the body. Lb exhibits adaptogenic, hepatoprotective, neuroprotective, and immunomodulatory effects. It is useful in the treatment of metabolic disorders, inflammation, cardiovascular diseases, and visual impairment. Lb berries may help in the treatment of many dangerous diseases (oncology, heart attack, diabetes, obesity, etc.). Lb mitigates radiation injury through the regulation of immune function, gut microbiota, and associated metabolites. The pharmacological multi-target effects of Lb berries are due to the rich composition of biologically active substances (BAS) [4-25].

Lb berries include 18 amino acids (8 of which are essential), vitamins (A, B, E, and notably vitamin C content surpassing that of oranges), essential and trace minerals (Ca, K, Zn, Fe, Na, Cu, Mn, Mg, Ge, Se, among others), polysaccharides (LBP-1, 2, 3, 4), omega-3 and omega-6 fatty acids, carotenoids, flavonoids, and various other bioactive compounds [4-25].

Lb was firstly introduced to RA and Artsakh by us in 2013. We have developed hydroponic and soil biotechnologies for producing seedlings from Lb seeds and cuttings. We have also compiled a phytocollection “Diabefit” of 8 valuable medicinal plants (*Lycium barbarum* L., *Stevia rebaudiana* Bertoni, *Smilax officinalis* L., *Leonurus* L., *Sambucus nigra* L., *Mentha piperita* L., *Hypericum perforatum* L.), and fungus (*Canoderma lusidum*) [26-29].

Ultra-microelements, (such as Se (selenium)) in the human body are important for the prevention of numerous diseases, including chronic and viral infections, and reducing the risk of their occurrence. Hydroponics can control the chemical composition of plant materials used in food by changing the composition of the nutrient solution that nourishes plants. Se is a component of more than 30 essential BAS that are found in human and animal organisms and is an active participant in various metabolic processes. In organisms, Se performs numerous protective functions that strengthen the immune system. It is known that Se deficiency is associated with the prevalence of various diseases, such as cardiovascular diseases, stroke, and cancer, which affect 60-80% of the population. The World Health Organization (WHO) recommends that adults consume 55–70 µg of Se daily [30].

Se deficiency in biogeocenosis is considered a serious problem. The Se content in agroecosystems ranges from 10 to 1100 µg/kg, depending on the chemical composition of the soil in different regions. In different ecosystems, plants differ in their ability to accumulate Se. To enrich plants with Se, the Se uptake of different plant species (parsley, radish, dill, lettuce, garlic, and wheat) was studied in different countries. The average Se content in plants of different populations is up to 100 µg/kg. According to our studies, *Stevia rebaudiana* Bertoni contains 130 µg/kg Se in soil conditions, and 43 µg/kg in hydroponics [29]. Amounts of Se in the range of less than 1 mg/kg are beneficial for plants, but higher concentrations can lead to various toxic symptoms, such as leaf necrosis, chlorosis, plant wilting, premature reduction in protein synthesis, and even lead to plant death [31-39].

Considering the above considerations, we studied the effect of exogenous addition of Se to the nutrient solution as well as foliar nutrition of plants with an aqueous solution of Se, on the productivity and accumulation of Se and BAS in the plant raw material of Lb under the conditions of hydroponics and soil in the Ararat valley. The obtained data may have a special practical significance, as they may become the basis for the development of biotechnology to produce selenium-enriched plant raw materials.

MATERIALS AND METHODS

The research was carried out in 2021-2023 in IHP in the Ararat Valley. The Ararat valley is located in Armenia, at an altitude of about 850-900 m above sea level. It has a dry continental climate, and the annual average air temperature is 11.0 - 11.8ºC [40]. IHP soil is rich in phosphorus and potassium and has about 1.5-2.5% humus. Soil plants were irrigated with artesian water.

In hydroponics, plants were nourished with a nutrient solution suggested by Davtyan [41], which was prepared using artesian water. The mixture of gravel with volcanic slag in a 3:1 ratio was used as a substrate. The particles of gravel and volcanic slag ranged in diameter from 3 to 15 mm. The substrate was disinfected with a 0.05% solution of $KMnO_4$ before the Lb planting.

Table 1: Scheme of scientific experiments

Version	Version description
1.	Control - hydroponic plants grown in a nutrient solution without the exogenous addition of Se
2.	Plants grown in a nutrient solution containing 1.0 mg/L of Se, supplemented with foliar nutrition using a 0.001 % Se solution
3.	Plants cultivated in a nutrient solution containing 1 mg/L of Se, along with foliar nutrition using a 0.005 % Se solution
4.	Plants grown in a nutrient solution supplemented with foliar nutrition using a 0.001 % Se solution
5.	Plants cultivated in a nutrient solution supplemented with foliar nutrition using a 0.005 % Se solution
6.	Nutrient solution + 1mg/L Se
7.	Control-soil, natural Se content in soil= 40 µg/kg
8.	Plants grown in soil supplemented with foliar nutrition using a 0.005 % Se solution

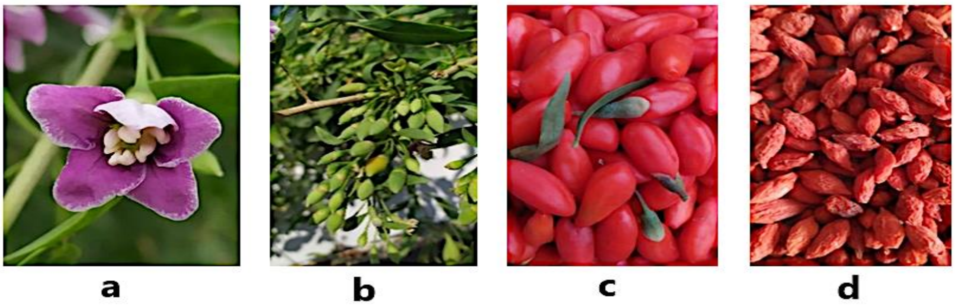


Figure 1: Flower of Lb (a), berries unripe (b), fresh (c) and dried (d)

During the vegetation period, biochemical measurements and a range of biochemical analyses were done. The content of vitamin C in fresh biomass was determined according to Yermakov [42]. The content of b-carotene was estimated according to Sapozhnikov [43]. Active substances in the extractable fraction were measured according to SPRF XIII [44], and flavonoid content was determined according to Georgievskiy [27,45].

The investigated air-dried material is pre-treated to obtain selenite ions (SeO_3). The ignition is conducted in an alkaline environment to prevent the loss of volatile forms. Further determination of Se mass concentration is performed using the GOST-19413-89 method. This standard establishes a fluorescent determination method. The method is based on the interaction of selenite ions with the reagent 2,3-diaminonaphthalene in an acidic environment, forming a compound called 4,5-benzopyrazoselenol. This compound is extracted with hexane and exhibits yellow-red fluorescence, the intensity of which is measured by a fluorimeter. To achieve this, hydroselenide ions and Se from organic compounds are converted into selenite ions by treating them with a mixture of nitric and hydrochloric acids. The measurement range is 0.1-5.00 µg/L [46-47].

Statistical Analysis: The obtained data were statistically analyzed using GraphPadPrism8, Excel, and according to Dospekhov [48], with each plant measurement repeated 3 times (n=3).

RESULT AND DISCUSSION

In case of soilless production, a mechanized and automated closed system is used for irrigation and application of mineral fertilizers. In this case, some environmental abiotic factors are regulated in a controlled hydroponics environment. It contributes to the improvement of the nutritional and water-air regime of crops, which ensures high biological efficiency of crops (Figures 2-7).

It is known that the accumulation of Se in plants mainly depends on several factors: the type of plant, the properties of the soil (type, acidity, humus content, and the presence of mineral elements), the chemical form of Se in the soil, its content, the way plants are treated with Se, ambient temperature, and humidity. According to literature data, the biological accumulation coefficient (BAC) of Se for plants in the

$$(BAC = \frac{Se\ content\ in\ the\ plant}{Se\ content\ in\ the\ soil})$$

soil ranges from 0.2-0.6 [31-39]. In the case of foliar nutrition of plants with a 0.005 % solution of Se in the gray desert soils of IHP territory (version 8), the BAC ranges between 1.1-1.5 in berries and 1.2-1.7 in leaves, and in hydroponics (version 2, 3, 6) it is between 0.038-0.06 in berries and 0.04-0.08 in leaves (Figure 2). It is obvious that in the soil plants the content of Se is higher by 25-28 times in berries and by 21-30 times in leaves, compared with the same rates of hydroponic plants. It should be noted that Clark Se in the world's soils is 400 µg/kg [35]. Although the natural Se content in the IHP soil is insufficient (Se = 40 µg/kg), the limit of Se concentration in berries and leaves is BAC > 1. This is consistent with the literature data, where it was shown that in the case of insufficient content of Se in black soil (Se = 100 µg/kg), corn and sunflower accumulated 107 µg/kg and 104 µg/kg of Se, respectively, and the BAC values were 1.07 and 1.04 respectively [38].

Foliar nutrition of plants with a 0.005% water solution of Se contributed to the increase of BAC of Se in berries and leaves. In hydroponics it resulted in 1.6 and 2.0 times increase (version 3), and in soil by 1.4 and 1.4 times (version 8), respectively.

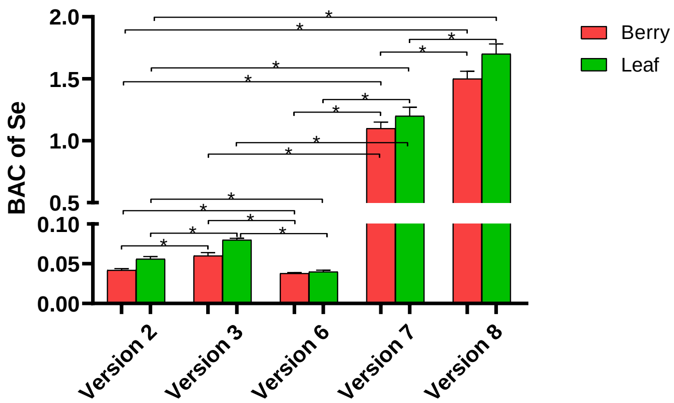


Figure 2: BAC of Se in berries and leaves of Lb in the soil plants and hydroponic plants. * - p<0.05.

It was found that the foliar nutrition of plants with a 0.005% water solution of Se in the soil contributed to an increase in the content of Se in berries and leaves (1.4 times), (Figure 3a) and an increase in the yield of berries (+15.0 %), (version 8), (Figure 3b). The exogenous addition of 1.0 mg/L of Se to the nutrient solution and the foliar nutrition of plants with a 0.005 % water solution of Se led to an increase in the concentration of this trace element in the berry by 2.0 times and in the leaves by 2.2 times, which had a positive effect on the yield of berries (+12 %) (version 3). In hydroponics and soil, the content of Se in leaves of LB exceeded the same index of fruits: in hydroponics by 1.2-1.3 times, and in soil by 1.1 times. The received data are consistent with the literature data, supporting Se had a positive effect on plant yield [39].

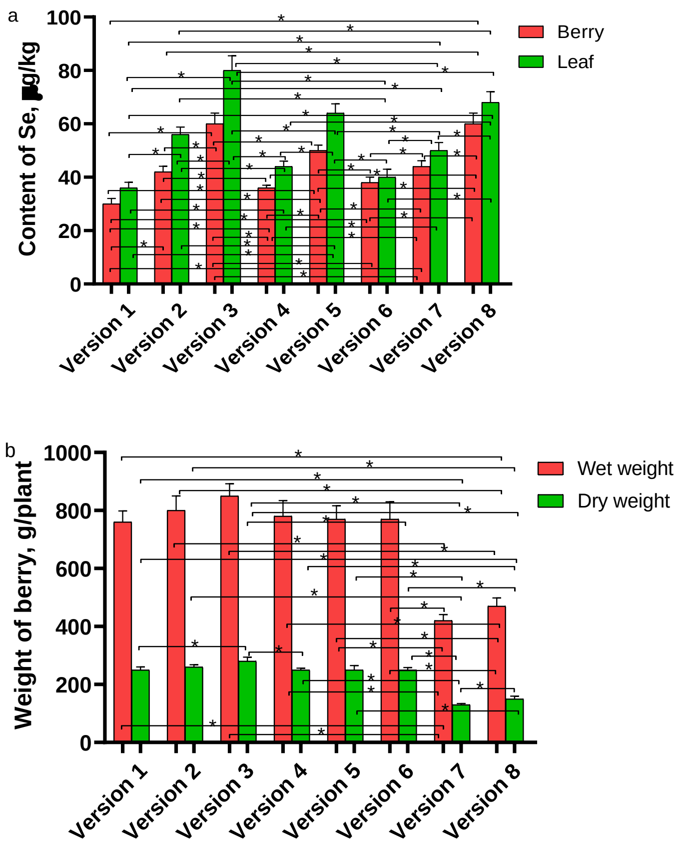


Figure 3: The influence of the nutrition by Se on its content in berries and leaves (a) and on berry yield (b) of Lb in hydroponics and soil. For the dry weight of the plant $LSD_{0,05}$ =14.5 g [48]. * - p<0.05.

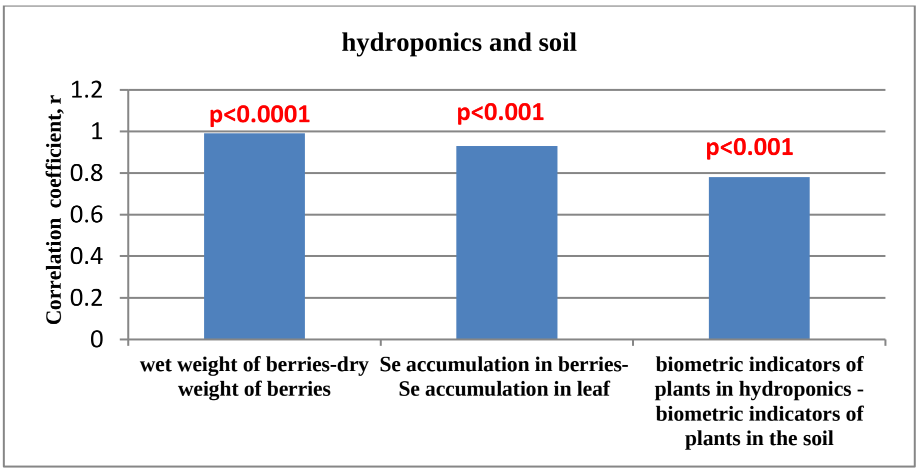


Figure 4: Correlation coefficients (r) between agrochemical indices of Lb plants in hydroponics and soil.

Positive strong comparative connections between plant indicators were observed (wet weight of berries - dry weight of berries couple r = 0.99 ± 0.056; t_{actual} = 17,6; Se accumulation in berries - Se accumulation in leaf couple r = 0.93 ± 0.14; t_{actual} = 6,6; biometric indicators in soil – biometric indicators in hydroponics couple r = 0.78 ± 0.25; t_{actual} = 3,1) (Figure 4) [48]. Since t_{actual} = 17,6; 6,6; 3,1 > $t_{theoretical\ 0.5}$ = 2.31, the correlation between the agrochemical indices is considered significant.

It has been established that the growing environment also has a significant impact on the synthesis of some BAS (extractive substances, flavonoids, vitamin C, β - carotene, and tannin) in Lb plants. The content of phenolic acids in the berries and leaves of Lb remained the same in hydroponics and soil. (Figures 5-7).

Lb berries in hydroponics are superior to soil Lb berries in flavonoids (1.8-2.3 times), vitamin C (1.7-2.1 times), b-carotene (1.5-1.8 times), and tannin content (1.1-1.3 times) (Figure 6a and Figure 7a). It was found that in the case of exogenous addition of Se to the nutrient solution in the amount of 1 mg/l in hydroponics and exfoliation of plants with a 0.005% solution of Se (version 3), in berries and leaves, the content of flavonoids (1.3 and 1.3 times), vitamin C (1.2 and 1.2 times), b-carotene (1.4 and 1.5 times), and tannins (1.1 and 1.1 times) increased. The obtained data partially coincides with the literature data [38]. In hydroponics, the leaves of Lb exceed the berries in the content of flavonoids (2.0 times), phenolic acids (5.0 times), and tannins (1.6 times) (version 1) (Figure 6a and Figure 6b). Berries of Lb exceed the leaves in terms of the content of extractive substances and β-carotene: 2.2 and 1.8 times (version 7), in hydroponics: 1.5 and 1.8 times (version 1) 1.6 and 2.2 times (version 3) (Figure 5, Figure 7a, and Figure 7b). In hydroponics, the leaves of Lb exceeded the berries in the content of flavonoids (2.0 times), phenolic acids (5.0 times), and tannins (1.6 times), (version 1) (Figure 6a and Figure 6b). According to Figure 6b, the content of flavonoids in the leaves of Lb in hydroponics (version 3) is 23.0 mg/g. This is in line with the literature data, according to which the same indicator is 21.25 mg/g in Lb leaves [4].

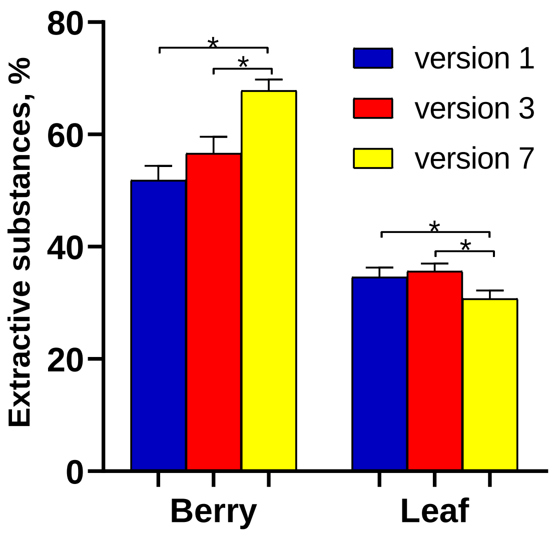


Figure 5: The content of extractive substances in Lb berries and leaves in hydroponics and soil in different study versions. * - p<0.05.

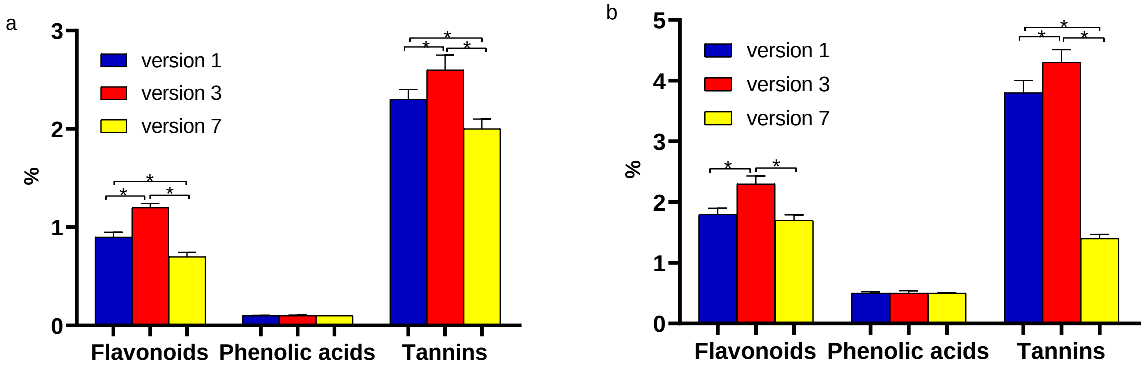


Figure 6: The content of BAS in the Lb berries (a) and leaves (b) of Lb in hydroponics and soil in different study versions. * - p<0.05.

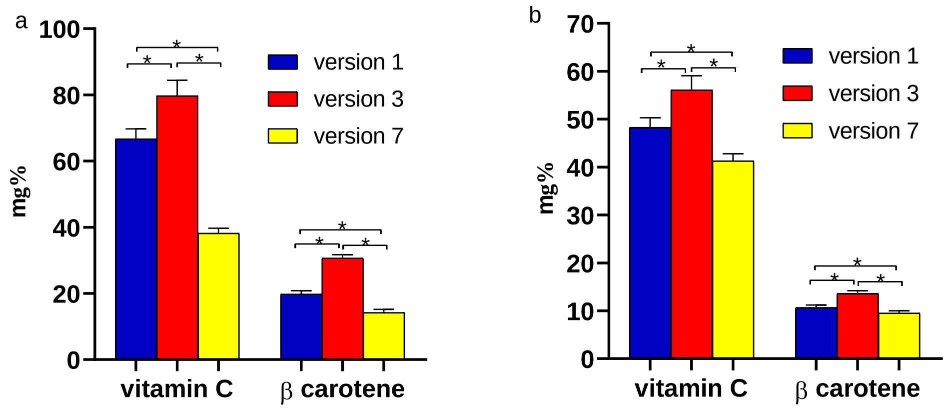


Figure 7: The content of vitamin "C" and β -carotene in Lb berries (a) and leaves (b) in hydroponics and soil in different study versions. * - p<0.05.

CONCLUSION:

In the hydroponic and soil culture Lb, foliar nutrition of leaves with a 0.005% aqueous solution of Se as well as the simultaneous exogenous addition of 1.0 mg/L Se to the nutrient solution contributed to the accumulation of Se and BAS in leaves and berries and increased productivity. Moreover, the influence of Se was more effective in hydroponics.

A practical suggestion: To obtain Lb plant material rich in Se, the hydroponic method of growing plants is preferred. In this case, 1.0 mg/L Se is added exogenously to the nutrient solution and the plants are nourished with foliar nutrition by the 0.005% aqueous solution of Se.

Abbreviations: BAC: biological accumulation coefficient, BAS: biologically active substances, IHP: Institute of Hydroponics Problems, Lb: *Lycium barbarum*, LBP: Polysaccharides of *Lycium barbarum*, LSD: least significant difference, SP: State Pharmacopoeia, RA: Republic of Armenia.

Authors contribution: All authors contributed to this study.

Competing interests: The authors declare no conflict of interest.

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