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The effect of different growth conditions on the content of bioactive compounds and gross β-radioactivity of some wild edible plants in the Republic of Armenia

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ABSTRACT

Background: Nowadays, people prefer using edible plants over synthetic drugs for the prevention and treatment of diseases. These plants are rich in bioactive compounds (BC), such as vitamins, carotenoids, proteins, carbohydrates, organic acids, flavonoids, tannins, and more. Humans have used them for thousands of years as both food and medicine. Through the rational use of edible plants, people aim to enhance the body's immunity and prevent diseases. In this context, the need to obtain edible plants that are both rich in BC and radioecologically safe has become an urgent concern.

Objective: To identify the optimal conditions for obtaining BC-rich radioecologically safe plant products, we studied the comparative characteristics of BC (protein, tannins, flavonoids) content and their gross β-radioactivity in several wild edible plants-WEP (sickleweed - *Falcaria vulgaris* Bernh., eryngium - *Eryngium caucasicum* Trautv., and rhubarb - *Rheum rhabarbarum* L.) under outdoor hydroponics and soil cultivation conditions. The research was conducted at the Institute of Hydroponics Problems (IHP) in the Ararat Valley; an area located within a 30 km radius of the Armenian Nuclear Power Plant (ANNP).

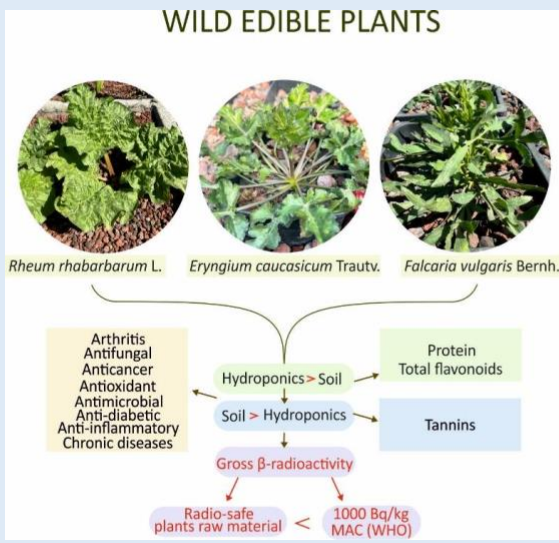
Methods: Total protein content was assessed using the Kjeldahl method, and tannin content was determined using a titrimetric method, while the total flavonoid content was quantified using a spectrophotometric method. Gross β-radioactivity of samples was defined with radiochemical methods through the radiometer UMF-1500 with low background.

Results: The cultivation method (hydroponics, soil) did not significantly affect the content of total flavonoids and tannins in sickleweed leaves. In hydroponics, the protein content in sickleweed increased by 1.3 times, while the flavonoid content in eryngium leaves and the tannin content were 1.2 times higher than those in soil-grown plants. The concentration of the nutrient solution (0.75N, 1.0N, 1.25N) significantly affected the flavonoid and tannin content in the petioles of rhubarb. The minimum flavonoid and tannin content in rhubarb petioles was recorded at 0.75N (0.2% and 2.1%, respectively), while the maximum content was observed at 1.25N (0.4% and 2.8%, respectively). Food plants, in order of gross β-radioactivity levels in hydroponics and soil, form the following descending order: sickleweed > eryngium > rhubarb. The gross β-radioactivity of plants varied between 780-910 Bq/kg in hydroponic systems, compared to 650-760 Bq/kg in soil.

Novelty: This study is the first to integrate biochemical profiling of BC with radiological safety assessment in WEP (sickleweed, eryngium, and rhubarb) cultivated under hydroponic and soil conditions. It demonstrates that hydroponics not only enhances protein, flavonoid, and tannin yields but also ensures radio-ecological safety, confirming WEP as promising functional food ingredients and medicinal resources.

Conclusion: In the raw materials of WEP, the content of certain BC in hydroponics exceeded that in soil. The raw materials of plants grown under different conditions are considered radio-ecologically safe because their gross β-radioactivity does not exceed the radio-ecological safety threshold set by the World Health Organization (< 1000 Bq/kg). Regardless of cultivation conditions, edible plants can be used as a functional food.

Keywords: *Falcaria vulgaris* Bernh., *Eryngium caucasicum* Trautv., *Rheum rhabarbarum* L., protein, tannin, total flavonoids, radio-ecological safety.



Graphical Abstract: The effect of different growth conditions on the content of bioactive compounds and gross β-radioactivity in some edible plants in Ararat Valley

INTRODUCTION

During the operation of nuclear reactors, the biosphere becomes polluted with numerous technogenic (^{137}Cs, ^{90}Sr, ^{89}Sr, etc.) and natural (^{40}K, ^{234}Th, ^{232}Th, etc.) radionuclides (RN). RN migrate through water, soil, and plants into the human body, causing dangerous diseases. According to the radioecological safety standards adopted by the World Health Organization (WHO), the gross β-radioactivity level in plant biomass intended for human consumption must not exceed 1000 becquerel per kilogram (Bq/kg). Consequently, monitoring the gross β-radioactivity levels in plant biomass intended for human consumption (wild edible plants (WEP), medicinal herbs, vegetables, fruits, etc.) and obtaining radio-safe plant materials remain current priority issues [1 - 7]. The present study was conducted at the Institute of Hydroponics Problems (IHP) in the Ararat Valley, located within a 30 km radius of the Armenian Nuclear Power Plant (ANPP) (Fig. 1).

Figure 1. The map of the studied areas of RA was taken from Google Maps: the distance between ANPP and the capital city Yerevan is 30 km.

WEP have been a staple part of the diet of many people since time immemorial. WEP have served humanity both as a rich source of nutrients and as medicinal herbs. WEP are considered functional foods due to their nutritional potential and role as sources of bioactive compounds (BC) (such as vitamins, protein, carotenoids, phenols, carbohydrates, essential oil, total flavonoids, terpenoids, alkaloids, tannins, and much more), that are vital for human health. Consequently, WEP exhibits a broad spectrum of therapeutic effects, including antimicrobial, antiviral, anti-inflammatory, antioxidant, anticancer, and antiparasitic properties.

Armenia, distinguished by its diverse landscapes and climates, is renowned for its rich variety of wild plants, with 3800 documented species. Of these, 380 species are used in traditional medicine, 90 species in scientific medicine, and 320 species as WEP. A critical concern is the significant decline in wild plant populations across Armenia in recent years, driven by the reduction of semi-deserts, the expansion of urban construction, and the ruthless use of natural resources. These anthropogenic stressors jeopardize both biodiversity and the ecosystem.

Consequently, the cultivation of WEP under hydroponic and soil-based systems is of critical importance from ecological, biodiversity conservation, public health, and economic perspectives. At present, the importance of WEP is also growing as a viable alternative to antibiotics. Approximately 85% of the population in developing countries primarily relies on plant-based medicines. When used according to traditional pharmacopeias, these plants demonstrate immunostimulatory and disease-preventive properties. The inclusion of BC-rich edible and medicinal plants in plant-based diets potentiates their therapeutic effects. The implementation of such plant-based diets by COVID-19 patients has demonstrated therapeutic anti-SARS-CoV-2 effects [8-38]. We have investigated the following WEP exhibiting both high nutritional value and BC-rich therapeutic properties.

Sickleweed (*Falcaria vulgaris* Bernh.), grows in Europe, Siberia, the Middle East, North Africa, North and South America, and the Caucasus. Grows in all floristic regions of Armenia. Sickleweed contains several BC, such as flavonoids, coumarins, tannins, proteins, and terpenes, which contribute to its potential medicinal properties. These compounds exhibit antioxidant, anticancer, anti-inflammatory, and antimicrobial activities, suggesting that the plant may play a role in protecting against oxidative stress and inflammation-related diseases. Tannins are known for their astringent properties, which allow them to bind and precipitate proteins, making them effective in many medical and health applications. Sickleweed may have potential applications in functional food ingredients aimed at improving digestion, wound healing, and reducing inflammation in general [28-31].

Eryngium (*Eryngium caucasicum* Trautv.), commonly known as Caucasian eryngo, shows potential as a functional food due to its BC and health benefits. Rich in BCs. These compounds can help combat oxidative stress, support digestive health, and enhance immune function, making eryngium a promising addition to functional foods aimed at improving overall well-being. Its anti-inflammatory effects may also contribute to the prevention of chronic diseases such as cardiovascular disease and arthritis. In addition, the plant's antimicrobial and antifungal properties may support gut health and promote a balanced microbiome, potentially improving digestive and immune function. Eryngium may find a place in the development of functional foods intended for health maintenance and disease prevention [32-35].

Rhubarb (*Rheum rhabarbarum* L.) is a species of flowering plant in the buckwheat family, native to southern Siberia and northern and central China. Rhubarb contains several BCs that contribute to its medicinal properties. The main anthraquinones, such as rhein, emodin, and chrysophanol, have anti-inflammatory, antioxidant, antimicrobial, and anticancer effects, while rhein also has liver-protective potential [36-38].

Our study aims to identify optimal conditions for obtaining high-quality, BC-rich, and radio-safe plant biomass from the aforementioned WEP (such as cultivation methods, types of substrates, concentration of nutrient solution, and radio-ecological zones). For this purpose, the comparative characteristics of WEP BC content and gross β-radioactivity under hydroponic and soil cultivation conditions were studied.

MATERIALS AND METHODS

Study Area and Conditions: The studies were conducted in the experimental field of the IHP under outdoor hydroponic and soil cultivation conditions in the Ararat Valley. The Ararat Valley is located at an altitude of approximately 850-900 m above sea level. The climate in this region is highly continental, with an average annual temperature of 11.0-11.8 °C, relative humidity of 40%, and average annual precipitation of 200-300 mm [39].

In hydroponics, the plants were nourished with Davtyan's nutrient solution (N 200 mg/L, P 65 mg/L, K 350 mg/L, pH 5.8-6.5, EC 1.2-1.3 mS/cm [40]. The hydroponic plants were nourished with the nutrient solution 1-2 times per day, while the soil-grown plants were irrigated with artesian water. The soil contained 1.5-2.5% humus and was rich in phosphorus and potassium. In soil cultivation, all necessary agrotechnical practices were maintained.

Biochemical and Radiochemical Measurements: The plants' tannin content was determined using a titrimetric method [41]. The total flavonoid was quantified using a spectrophotometric method [40]. Total protein content was assessed using the Kjeldahl method. [42]. Gross β-radioactivity of samples was defined with radio-chemical methods through the radiometer UMF-1500 with low background [43]. Each plant measurement was repeated 3 times (n=3).

Statistical Analysis: The statistical analysis was done using GraphPad Prism 8, and Excel. $*p < 0.05$ was considered statistically significant.

Plant Sampling: Plant samples were collected from the edible parts of plants: from aboriginal 1-year-old sickleweed, from 1- and 2-year-old eryngium, that was introduced from the Islamic Republic of Iran (IRI), and 3-year-old mother rhubarb, which was introduced from Norway. Samples were also taken from wild eryngium growing in the surrounding soils of the cities of Sari, Rasht, and Ramsar (IRI).

RESULTS

The results of biochemical analysis of WEP grown under different conditions are presented in Figures 2-9. However, under the same conditions, the content of tannins and total flavonoids in sickleweed remained unchanged (Fig. 2a, 3a). In the hydroponic cultivation of sickleweed, the protein content in plant biomass was 1.3 times higher than in soil-grown plants (Fig. 4a). Moreover, in hydroponic cultivation, sickleweed outperformed soil-grown plants in output of tannins, total flavonoids, and protein by 1.8, 1.8, and 2.3 times, respectively (Fig. 2b, 3b, 4b).

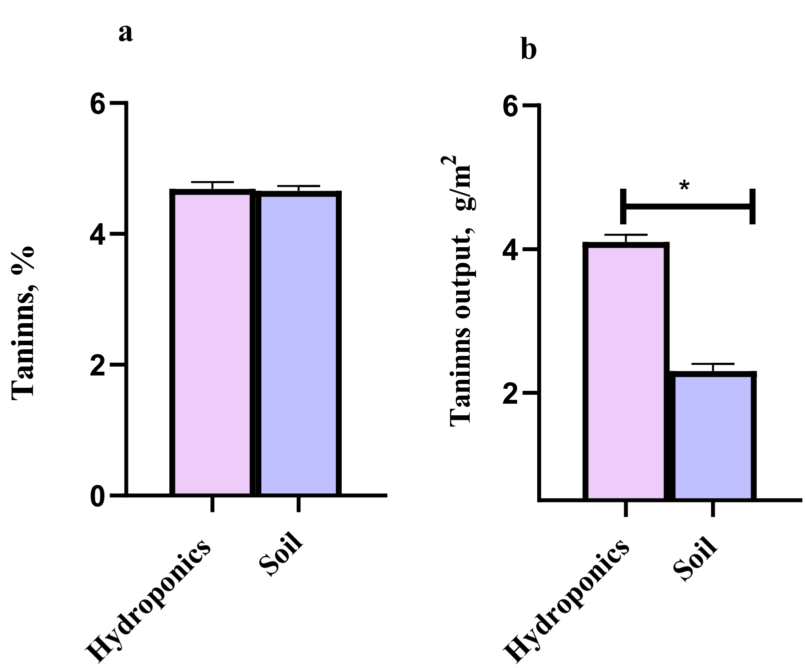


Figure 2. Tannin content (a) and output (b) of sickleweed in hydroponic and soil conditions: $*p < 0.02$.

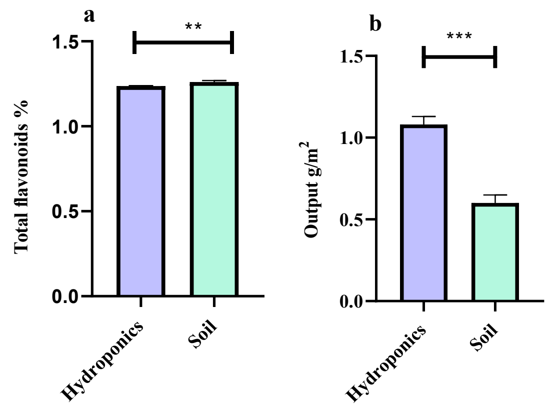


Figure 3. Total flavonoid content (a) and output (b) of sickleweed in hydroponic and soil conditions: **p<0.002, ***p<0.0003.

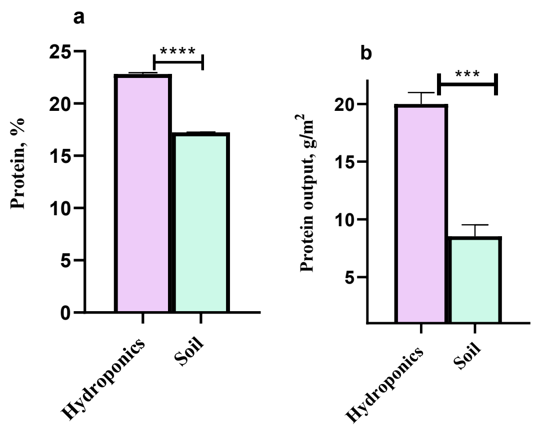


Figure 4. Protein content (a) and output (b) of sickleweed in hydroponic and soil conditions: ***p<0.0001,****p<0.0001.

Comparative analysis of 2-year-old eryngium plants revealed significant differences in leaf phytochemical content between hydroponic and soil cultivation conditions. Hydroponically grown eryngium leaves demonstrated 1.2 times higher concentrations of both total flavonoids and tannins compared to soil-grown ones (Fig. 5a, b).

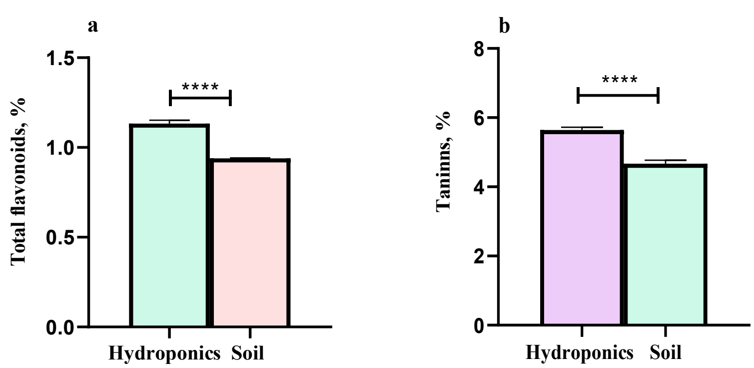


Figure 5. Total flavonoid (a) and tannin (b) content in eryngium (2- year-old) in hydroponic and soil conditions: ****p<0.0001.

It should be mentioned that 2-year-old hydroponically cultivated eryngium exhibited 1.4 and 1.3 times higher concentrations of total flavonoids and tannins, respectively, compared to 1-year-old plants.

It was found that the concentration of the nutrient solution affected rhubarb BC content. When rhubarb was cultivated hydroponically in a 1.25N nutrient solution, the total flavonoid content and output in the petioles exceeded those of plants grown in 0.75N and 0.1N nutrient solutions, as well as in soil, by 1.6, 1.1, 1.7 times and 1.7, 1.1, 3.3 times, respectively (Fig. 6a, b).

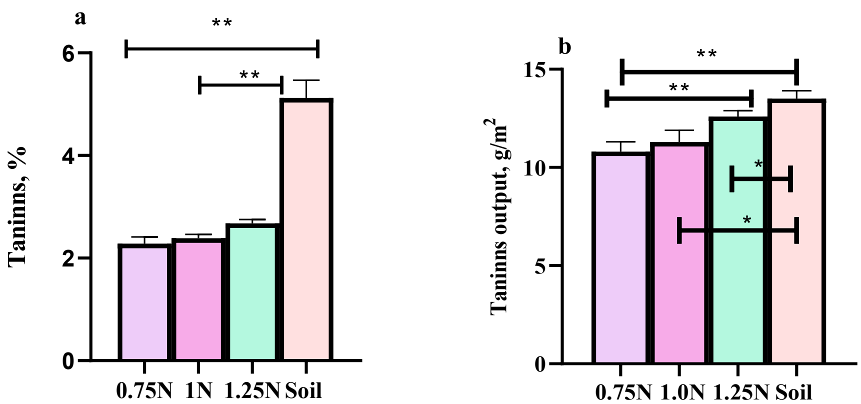


Figure 6. Tannin content (a) and output (b) of rhubarb petioles in hydroponic and soil conditions: *p<0.03, **p<0.006.

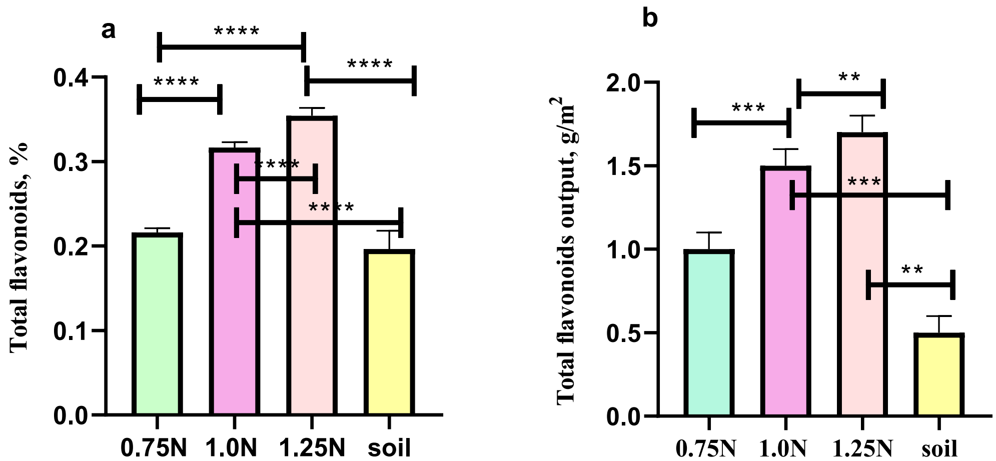


Figure 7. Total flavonoid content (a) and output (b) of rhubarb petioles in hydroponic and soil conditions: **p<0.001, *** p<0.0003, **** p<0.0001.

In contrast, soil-grown rhubarb petioles exhibited 2.2, 2.1 and 1.9 times higher tannin content, along with 1.2, 1.2 and 1.1 times greater tannin output compared to plants nourished with 0.75N, 1.0N, and 1.25N nutrient solutions, respectively (Fig.7 a, b).

When cultivating rhubarb hydroponically using different substrates, significant variations were observed in petiole concentrations of total flavonoids and tannins. Moreover, based on their content of total flavonoids in rhubarb petioles, the different substrates form the following descending order: volcanic slag>gravel>volcanic slag+gravel mixture. Regarding tannins content, the order is: gravel> volcanic slag> volcanic slag+gravel mixture (Fig. 8a, b).

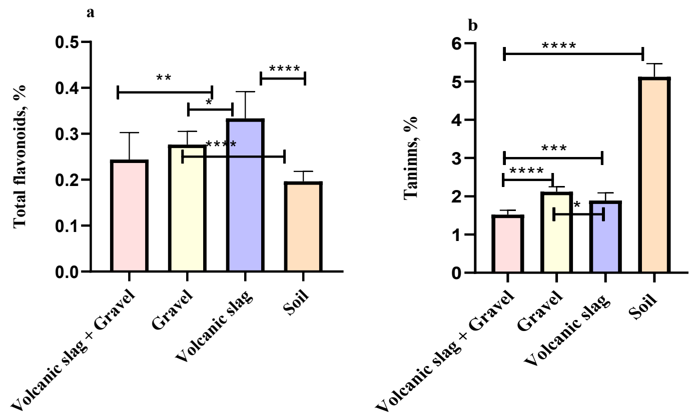


Figure 8. Total flavonoid (a) and tannin (b) content in rhubarb petioles grown in different substrates under hydroponic and soil conditions: *p<0.02, ** p<0.008, ***p<0.0004, ****p< 0.0001.

Thus, when cultivating rhubarb hydroponically using volcanic slag substrate, petiole total flavonoid content increased 1.2, 1.4, and 1.7 times compared to gravel substrates, volcanic slag + gravel mixture, and soil cultivation, respectively. In soil-cultivated rhubarb, petiole tannin content exceeded that of hydroponic ones using gravel, volcanic slag, and volcanic slag + gravel mixture substrates by 2.4, 2.7, and 3.4 times, respectively.

Regarding total flavonoid content, the edible plants maintained the same descending order in both hydroponic and soil cultivation: sickleweed>eryngium> rhubarb. Furthermore, sickleweed showed 1.1 and 1.3 times higher flavonoid concentrations than eryngium, and 4.1 and 6.3 times higher concentrations than rhubarb in hydroponic and soil cultivation conditions, respectively. Based on tannin content, the edible plants form the following descending orders: in hydroponics: eryngium>sickleweed>rhubarb, and in soil: rhubarb>sickleweed=eryngium. In hydroponic systems, eryngium contained 1.2 and 2.1 times higher tannin levels than sickleweed and rhubarb, respectively. In soil cultivation, rhubarb showed 1.1 times greater tannin content than both sickleweed and eryngium.

The results of radiochemical analysis for edible plants cultivated under different conditions are presented in Figures 9-12. In hydroponics the gross β-radioactivity of edible plants ranged between 780-910 Bq/kg, while in soil it ranged between 650-760 Bq/kg (Fig. 9).

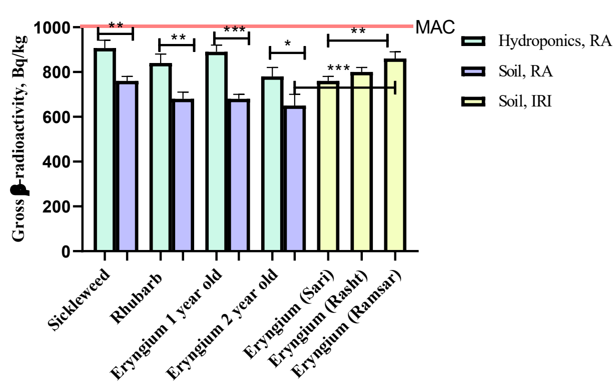


Figure 9. Gross β-radioactivity of edible plants in hydroponic and soil conditions in Armenia and IRI: *p<0.02, **p<0.003, ***p<0005.

Hydroponically grown sickleweed, rhubarb, and eryngium exceeded soil-grown ones in gross β-radioactivity by 1.2, 1.2, and 1.2-1.3 times, respectively. In both hydroponic and soil cultivation, the edible plants formed the following descending order of gross β-radioactivity levels: sickleweed>rhubarb>eryngium**.** Wild eryngium leaves collected from soils surrounding Sari, Rasht, and Ramsar cities in IRI exhibited gross β-radioactivity levels ranging from 760-860 Bq/kg. Notably, wild eryngium from Ramsar soils showed 1.3- and 1.3-times higher radioactivity than 1- and 2-year-old eryngium cultivated in soils surrounding the IHP, respectively. In hydroponics, 1-year-old eryngium exhibited 1.1 and 1.4 times higher gross β-radioactivity compared to 2-year-old plants in hydroponic and soil cultivation, respectively**.** Sickleweed exhibited 1.1 and 1.2 times higher gross β-radioactivity than rhubarb and eryngium in hydroponics, and 1.1- and 1.1-1.2-times higher levels in soil cultivation, respectively.

It was found that concentration of nutrient solution did not significantly affect gross β-radioactivity of the rhubarb petioles (Fig. 10). The rhubarb petioles by RN content are more inferior than the rhubarb leaves in both hydroponics and soil cultivation (Fig. 11). In hydroponics plants leaves and petioles grown in the volcanic slag + gravel mixture are 1.1-1.4 times inferior by the β-radiating RN content than those of both gravel and volcanic slag grown plants (Fig. 11**)**.

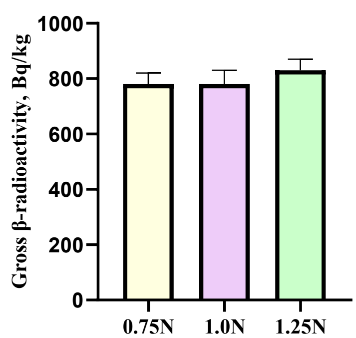


Figure 10. Gross β-radioactivity of rhubarb petioles in hydroponic and soil conditions.

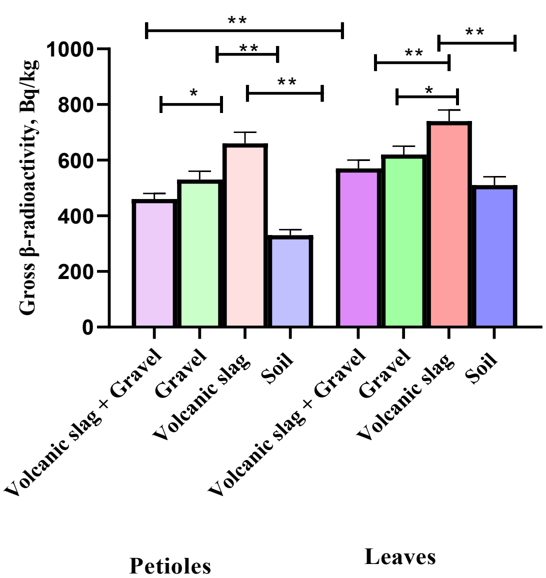


Figure 11. Gross β-radioactivity of rhubarb leaves and petioles in hydroponic and soil conditions: *p<0.05, **p<0.005.

DISCUSSION

This study demonstrates that biotic and abiotic factors in the Ararat Valley significantly influence both BC profiles and β-radiating technogenic and natural RN accumulation in WEP biomass under hydroponic and soil cultivation conditions. Hydroponics enhances both the biosynthesis of BC in plants and improves crop productivity by providing optimal conditions for normal plant growth and development [44]. Thus, applying hydroponic biotechnology to WEP cultivation through optimized substrates, nutrient solution concentrations, and other parameters can simultaneously enhance health-beneficial BC content in plant biomass while reducing harmful β-radiating technogenic and natural RN.

Hydroponically cultivated WEP exhibit higher concentrations of BC, including protein, total flavonoids, and tannins, compared to soil-grown ones. This demonstrates the superior therapeutic potential of hydroponic plants over soil-cultivated ones. Specifically, the biosynthesis of protein in sickleweed, total flavonoid and tannin content in eryngium, and total flavonoid content in rhubarb grown in volcanic slag substrate were more intensive in hydroponics than in soil. Rhubarb was an exception, where tannin biosynthesis was more intensive in soil than in hydroponics. Although tannin and total flavonoid biosynthesis in sickleweed proceeded with equal intensity in both soil and hydroponics, the output of tannins and total flavonoids in hydroponically grown sickleweed exceeded those in soil-grown plants by 1.8 and 1.8 times, respectively. The presence of BC in sickleweed, eryngium, and rhubarb confirms their multifunctional therapeutic effects, including antiviral, antioxidant, anti-inflammatory, antidiabetic, immunomodulatory, and other beneficial properties**.** Sickleweed can be included in plant-based diets as a protein source to help meet daily human protein requirements. Human consumption of sickleweed as a food source also contributes to reducing blood glucose levels. This is supported by the study of Jafar et al. [28], which demonstrates that sickleweed possesses potential antidiabetic effects. This phenomenon results from the bioactive effects of tannins and total flavonoids contained in sickleweed. The literature indicates that tannins and flavonoids in plants, due to their antioxidant activity, contribute to the neutralization of harmful free radicals, thereby protecting cells from oxidation [10, 36].

It has been revealed that within a 30 km radius of the ANPP in the Ararat Valley, different cultivation conditions of certain plant species have a measurable influence on their gross β-radioactivity levels. Moreover, the gross β-radioactivity of plant species primarily depends on their biological characteristics such as their ability to selectively absorb mineral nutrients, duration of vegetation, shape, size, and anatomical structure of leaves, as well as the nature of root distribution in soil. Hydroponically grown edible plants show higher gross β-radioactivity than soil-grown ones, primarily because their nutrient solution contains more potassium (K) (350 mg/L) than soil [2]. As a result, in hydroponics, the roots of edible plants absorb K from the nutrient solution more intensively than from the soil. It is known that the gross β - radioactivity of crops is mainly connected with the content of K (thanks to ^{40}K), because ^{40}K has the greatest β- radioactivity than the rest of the RN. Thus, the radioactivity of ^{40}K is conditioned in 89.33% by β-radiation and in 10.67% by γ-radiation [2].

Our studies demonstrate that leafy vegetable crops (sickleweed, eryngium, rhubarb, lettuce, mizuna, kale, Chinese cabbage) exhibit relatively higher levels of gross β-radioactivity, whereas grain vegetable crops (corn, lentil, chickpea, bean) show lower gross β-radioactivity [4, 45]. Our research has established that cultivation method, nutrient solution concentration, and substrate type constitute significant factors influencing the gross β-radioactivity levels in food crops. The most radio-safe substrate for hydroponic cultivation of rhubarb is a mixture of volcanic slag and gravel. From a radio-safety perspective, rhubarb cultivation is preferable under hydroponic conditions using nutrient solutions with concentrations of 0.75N and 1.0N, along with a volcanic slag **+** gravel substrate. In addition, the petioles of rhubarb exhibit significantly lower radiological risk compared to its leaves.

Hydroponic plants can be considered more radio-safe than soil-grown plants, because in the gross β-radioactivity of various plant species (medicinal herbs, vegetables, tree fruits, etc.), the proportion of the most hazardous technogenic RN (^{90}Sr and ^{137}Cs) in hydroponics was 2.3-6.8%, while in soil it was 3.0-12.8%. That is, hydroponic plant biomass is more radiologically safe (1.3-1.9 times) than that cultivated in soil [40]. This is explained by the fact that the gross β-radioactivity of the nutrient solution (3.5 Bq/L) is significantly lower than the same indicator in soil (of 0-30 cm soil layer gross β-radioactivity=170 Bq/kg) [45]. Regardless of plant species and cultivation conditions, the obtained plant material can be considered radio-safe, as its gross β-radioactivity level complies with the radioecological standards (<1000 Bq/kg) established by WHO [1]. The cultivation of WEP in hydroponics is preferable to soil ones, both from BC production (with some exceptions) and from a radio-safety perspective.

CONCLUSION

Through controlled management of biotic and abiotic factors, WEP can be cultivated to produce high-quality plant biomass that is both rich in BC and radio-safe. Hydroponically cultivated WEP have shown significant potential for enhancing BC production while maintaining radio-safety, confirming their value as functional food ingredients and medicinal plants. The exception is soil-grown rhubarb, which exhibits higher tannin content and output compared to hydroponically cultivated plants**.** In the Ararat Valley, both hydroponically and soil-cultivated WEP as well as wild eryngium growing in the IRI are considered radio-safe, since the gross β-radioactivity of their plant biomass complies with WHO radioecological safety standards (<1000 Bq/kg).

List of Abbreviations: ANPP, Armenian Nuclear Power Plant; BC, bioactive compounds; Bq, becquerel; COVID-19, coronavirus disease; IHP, Institute of Hydroponics Problems; IRI, Islamic Republic of Iran; MAC, Maximum Allowed Concentration; RA, Republic of Armenia., RN, radionuclides; SARS, severe acute respiratory syndrome; WEP, wild edible plants; WHO, World Health Organization.

Authors' Contribution: All authors contributed to this study.

Competing interests: The authors declare no conflict of interest.

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