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The study of bioactive compounds and gross β-radioactivity of some grain vegetables and medicinal plants in outdoor hydroponic systems and soil culture in Ararat Valley

Laura Ghalachyan[1], Stepan Mairapetyan[1], Anna Tadevosyan[1], Lusya Hovhannisyan[1], Khachatur Mairapetyan[1], Anahit Hakobjanyan[1], Mahsa Daryadar[1], Ruben Siseryan[1], Anahit Tovmasyan[1], Aristakes Ghahramanyan[1], Artur Matevosyan[1], Anjelika Stepanyan[1], Armenuhi Asatryan[1], Gayane Shaboyan[1], and Anush Vardanyan[2*]

[1]Department of Plant Nourishment and Productivity, G.S. Davtyan Institute of Hydroponics Problems NAS RA, Republic of Armenia; [2]Departament of Tissue Culture, G.S. Davtyan Institute of Hydroponics Problems NAS RA, Republic of Armenia

***Corresponding Author:** Anush Vardanyan, PhD, Head of Tissue Culture Department, G.S. Davtyan Institute of Hydroponics Problems NAS RA, Noragyugh 108, Yerevan 0082, Armenia.

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ABSTRACT

Background: In the 21st century, nuclear reactors pose a significant threat to humanity. During operation, they release both natural (^{40}K, ^{234}Th, ^{232}Th, etc.) and technogenic (^{137}Cs, ^{90}Sr, ^{89}Sr, etc.) radionuclides (RN) into the biosphere, which can negatively affect human health. These RN enter agroecosystems through various transfer pathways. In hydroponics, the RN enters agroecosystems through a nutrient solution, then through the substrates, and finally through the plant. In soil, RN enters agroecosystems through irrigation from water to the soil and eventually to the plant. Through air basins, RN can also infiltrate hydroponic and soil-grown plants. Consequently, the accumulation of RN in plants poses health risks when consumed. Therefore, monitoring β-emitting RN in agricultural crops and medicinal plants is crucial to ensuring radio-safe plant materials.

Objective: This study aims to identify the optimal conditions for cultivating plants with a high content of bioactive compounds (BC), that are radioecologically safe. We investigated the β-emitting RN and BC content in various grain crop varieties, including lentil (Talini 6 – aboriginal, Flip 2007 3L – introduced from ICARDA), corn, chickpeas (Anush, Karin – aboriginal, Flip-07-44B – introduced from ICARDA), soybean (Menua – aboriginal), and medicinal plants introduced to Armenia: *Echinacea (E.) purpurea* (L.) Moench and *Lavandula (L.) angustifolia* Mill. These plants were grown in different hydroponic systems, including classical, water-stream, drip, and soil culture. The research was conducted at the

Hydroponics Experimental Station, located 30 km from the Armenian Nuclear Power Plant (ANPP). Based on our findings, we have developed practical recommendations to ensure the production of radioecologically safe plant material.

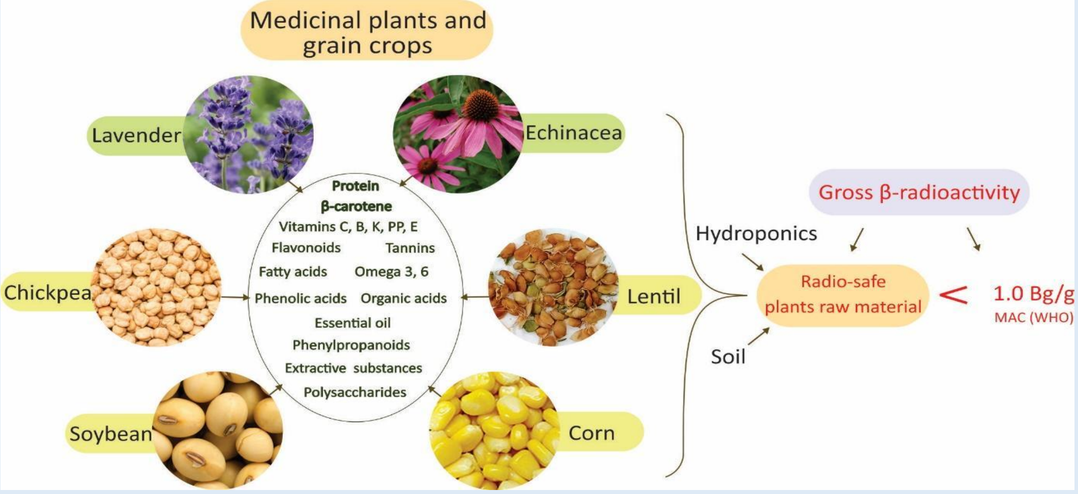
Methods: Total protein content was determined using the Kjeldahl method. Gross β-radioactivity level in plant samples was measured using UMF-1500 equipment.

Results: Protein content ranged from 9.7 to 31.5% in hydroponically grown grain crops and from 11.7 to 24.0% in soil-grown crops. Based on protein content, hydroponically grown crops ranked as follows: soybean>lentil (Flip 2007 3L)>lentil (Talini 6)>chickpea (Flip 07-44B)>chickpea (Karin)>corn. In soil-grown plants, the ranking differed: lentil (Flip 2007 3L)>soybean>lentil (Talini 6)>chickpea (Flip 07-44B)>chickpea (Karin)>corn. Gross β-radioactivity in classical hydroponic system grown grain crops ranged from 70-400 becquerels per kilogram (Bq/kg), while soil-grown grain crops exhibited radioactivity levels between 50-410 Bq/kg. Medicinal plants showed gross β-radioactivity values between 400-550 Bq/kg (hydroponically) and 260-370 Bq/kg (soil). The ranking for hydroponically grown crops based on gross β-radioactivity in their edible parts was as follows: soybean>chickpea (Anush)>chickpea (Karin)>chickpea (Flip 07-44B)>lentil (Talini 6)>lentil (Flip 2007 3L)>corn. For medicinal plants, the order was as follows: *E. purpurea*>*L. angustifolia*.

Novelty: For the first time in the Ararat Valley, optimal cultivation conditions for radio-safe crops and medicinal plants rich in BC (proteins, polysaccharides, phenols, essential oils, etc.) have been identified within a 30 km radius of the ANPP. These plants can be utilized for medicine and as ingredients for functional foods.

Conclusion: Both indigenous and introduced crops, along with medicinal plants cultivated under various hydroponic and soil conditions in the IHP (Institute of Hydroponics Problems) area of the Ararat Valley, have been confirmed as radioecologically safe and rich in BC. Their gross β-radioactivity did not exceed 1.0 Bq/g, confirming their suitability for consumption according to WHO guidelines.

Keywords: protein, essential oil, flavonoids, polysaccharides, fatty acids, radionuclides, radio-ecological safety.



Graphical Abstract: The study of bioactive compounds and gross β-radioactivity of some grain vegetables and medicinal plants

INTRODUCTION

Throughout Earth's history, flora and fauna have continually been exposed to natural radiation from cosmic and environmental sources. However, human activities have significantly increased radiation levels through the introduction of technogenic radionuclides (RN). Both natural RN (^{40}K, ^{234}Th, ^{232}Th, etc.) and (^{137}Cs, ^{90}Sr, ^{89}Sr, etc.) are released during nuclear reactor operations, infiltrating ecosystems and posing substantial health risks. These RN enter biological systems via biogeochemical pathways, such as "irrigation water to soil to plants" in agroecosystems and "nutrient solution to substrate to plant" in hydroponic systems. Additionally, RN can be absorbed by plants from the air basin. The extent of the health impact depends on the characteristics of the RN and the concentration, duration, and intensity of the exposure. According to World Health Organization (WHO) standards, the gross β-radioactivity of plants should not exceed 1.0 becquerel per gram (Bq/g). Therefore, the monitoring and control of β-emitting RN in vegetables and medicinal plants is of crucial importance to ensure the production of radio-safe raw materials that can also be used as ingredients for functional foods and medicine [1-5].

Hydroponic biotechnology offers a promising solution for cultivating high-quality, radio-safe plant materials rich in bioactive compounds (BC). By optimizing hydroponic systems, BC content can be maximized while maintaining radioecological safety and improving plant quality [6].

Edible and medicinal plants have historically been used in traditional Armenian medicine for their therapeutic benefits. Vegetables are valuable functional food ingredients due to their rich content of secondary metabolites such as vitamins, carotenoids, phenols, flavonoids, terpenoids, alkaloids, tannins, steroids, and essential oils. These compounds exhibit various biological properties, including antimicrobial, antioxidant, antiviral, anti-inflammatory, anticancer, antiparasitic, antihemolytic, antimutagenic, antifungal, and immunomodulatory characteristics.

Protein-rich crops such as lentils, chickpeas, corn, and soybeans are particularly important for human health, providing essential nutrients needed for bodily function. Additionally, plant proteins also provide amino acids necessary for human nutrition. More than 35,000 to 70,000 plant species have been evaluated for their medicinal and nutritional properties. Consuming plants rich in BC strengthens the immune system and reduces the risk of chronic diseases. Certain medicinal plants have demonstrated antiviral activity against herpes, influenza, HIV, hepatitis B and C, and SARS and MERS-CoV. The importance of medicinal plants was also highlighted during the prevention and treatment of COVID-19. Many studies have shown that vegetarians who consume more plant protein are less susceptible to COVID-19, so nutritionists recommend enriching the diet with protein-rich plant foods for optimal health [7, 29].

This study focuses on several protein-rich grain crops that serve as a source of healthy food and medicinal plants rich in BC.

Lentil (*Lens culinaris* Medik) is a highly nutritious plant with a protein content of up to 30%, second only to soybeans among cereals. It is considered an essential part of a healthy diet. Lentils are rich in fiber, folic acid, complex carbohydrates, and essential BC (flavonoids, polyphenols, saponins, tannins, etc.), contributing to their antioxidant and anti-inflammatory properties [7].

Chickpea (*Cicer arietinum* L.) is known for its nutritional density, which helps manage digestive disorders, hypercholesterolemia, type 2 diabetes, and cardiovascular diseases (CVD), while also exhibiting antioxidant, anti-inflammatory, antimicrobial, and anticancer properties [8].

Corn (*Zea mays* L.) and its by-products (cobs, roots, stems, etc), commonly used in food production, offer diuretic, hepatoprotective, antidiabetic, antioxidant, neuroprotective, and anticancer benefits [9].

Soybean (*Glycine max* (L.) Merr.) is rich in BC, particularly total saturated fatty acids (TSFA) and unsaturated fatty acids. The five key fatty acids (FAs) – oleic acid (OA), linoleic acid (LA, omega-6), linolenic acid (LNA, omega-3), palmitic acid (PA), and stearic acid- play an essential role in preventing cancer, diabetes, and CVD [10-11].

Echinacea (*Echinacea* (*E.*) *purpurea* (L.) Moench) is used in more than 240 medicines. Echinacea strengthens the immune system, serving as an anti-inflammatory and anti-infective agent for treating gastrointestinal, genitourinary, and respiratory disorders. It also reduces leukopenia by increasing lymphocyte and monocyte levels, thereby promoting blood cell recovery [12-13].

Lavender (*Lavandula* (*L.*) *angustifolia* Mill.) is a highly versatile medicinal plant used in the pharmaceutical, cosmetic, and food industries due to its high BC content. Lavender's essential oil possesses sedative, antidepressant, anti-inflammatory, antifungal, and antimicrobial properties, with strong activity against *E. coli*, *P. aeruginosa*, *P. mirabilis*, *K. pneumoniae*, *A. baumannii*, *S. aureus*, *E. faecalis*, and *B. subtilis* [14-15].

This research aims to identify the optimal factors (such as growing methods, hydroponic systems, radioecological zones, plant species, and varieties) required to produce high-quality, radio-safe plant materials. To achieve this, we performed a comparative study of BC content and gross β-radioactivity in several grain crops and medicinal plant species grown in different hydroponic systems (classical (CH), aquaponics (AH), water-stream (WSH), drip (DH)) and under soil conditions.

MATERIALS AND METHODS

Study Area and Conditions: The study was conducted at the IHP (Institute of Hydroponics Problems) experimental station in Yerevan, Armenia, and Darakert and Martuni, from 2021 to 2024, under outdoor hydroponic and soil conditions. Yerevan and Darakert are located in the Ararat Valley, while Martuni lies within the basin of Lake Sevan at an elevation of 1950m above sea level. In Martuni, the average temperature ranges from -6 °C to -14 °C in January and from 6 °C to 16 °C in August and September, with annual precipitation between 350 mm and 800 mm. The climate in the Ararat Valley is sharply continental. The average air temperature from July to August is 25-26 °C, with an average annual precipitation of 300 mm. During plant sample collection, the average air temperature was 26-30 °C in the Ararat Valley and 20-22 °C in Martuni (Fig. 1) [30].

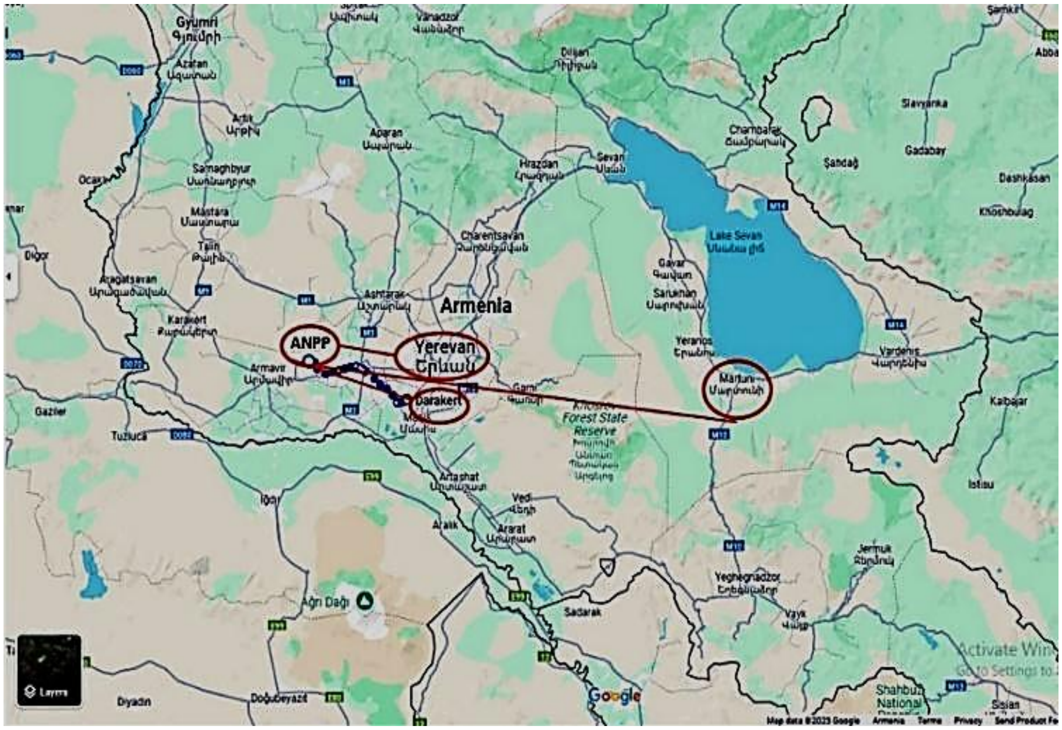


Figure 1. The map of the studied areas of RA was taken from Google Maps: the distance between ANPP and the capital city Yerevan is 30 km. The distance between ANPP and the village of Darakert is 22 km, while the distance between ANPP and the town of Martuni is 96 km.

Davtyan's nutrient solution (pH 5.8–6.5, EC 1.2–1.3 mS cm^{-1}; K 350 mg/L^{-1}, N 200 mg/L^{-1}, P 65 mg/L^{-1}) was used in hydroponics [6] and was prepared using artesian water. The hydroponic substrate consisted of gravel and volcanic slag with a 3–15 mm particle diameter. In CH, plants were nourished with the nutrient solution 1–2 times daily. In WSH, the nutrient solution was periodically pumped irreversibly as a stream to the rhizosphere. Under soil conditions, artesian water was used for irrigation.

Biochemical and Radiochemical Measurements: The total flavonoid and phenylpropanoid content in air-dried plant materials of *E. purpurea* was assessed using a spectrophotometric method, following the guidelines of the State Pharmacopeia XIV of RF [31]. Total protein was determined by the Kjeldahl method [32]. Sugar content was analysed using the Bertrand method [33]. The essential oil content in fresh plant raw material of *L. angustifolia* was estimated using the Ginzberg method [34]. Gross β-radioactivity of the samples was measured using a radiochemical method with a small background radiometer UMF-1500 [35]. The obtained radioactivity results were compared with MAC [1].

Plant Sampling: Plant samples were collected from the edible parts of grain crops, including corn; chickpea (varieties: "Anush" and "Karin" – aboriginal, and "Flip-07-44B" – introduced from ICARDA); lentil (varieties: "Talini 6" – aboriginal, and "Flip 2007-3L" – introduced from ICARDA); soybean (variety "Menua" – aboriginal) during the fruit ripening period. Samples of *E. purpurea* and *L. angustifolia* (above-ground parts) were harvested during the flowering period. Each plant measurement was performed in triplicate (n=3).

Statistical Analysis: Statistical analyses were conducted using GraphPad Prism 8, with a significance threshold of $p < 0.05$ (*).

RESULTS

The comparative analysis of key bio-pharmaco-chemical indexes in the studied plants, grown under different conditions, is presented in Figures 2–8. Various cultivation methods influenced the protein content in chickpeas, forming the following decreasing order for the Karin variety: soil > DH > WSH > CH, For the Flip 07-44B variety, soil > CH > DH > WSH (Fig. 2a). Soil-grown chickpeas of the Karin and Flip 07-44B varieties exhibited 1.1 – 1.3 times higher protein content compared to those cultivated in WCH, CH, and DH. For protein output, the Karin and Flip 07-44B varieties grown in WCH exceeded soil plants by 1.9 and 1.9 times, respectively, and those grown in DH exceeded soil plants by 1.5 and 2.1 times, respectively. In hydroponics, Karin variety plants surpassed soil-grown ones in protein output by 1.1 times. In contrast, no significant difference was observed in this indicator for Flip 07-44B (Fig. 2b). According to chickpea protein output, different cultivation methods resulted in the following decreasing order: WSH > DH > CH > soil. The Flip-07-44B variety had 1.1 times better protein output than the Karin variety in CH, 1.2 times in WSH, 1.7 times in DH, and 1.2 times in soil (Fig. 2b).

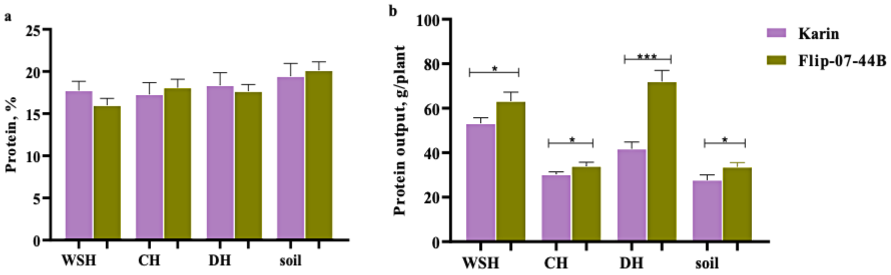


Figure 2. Protein content (a) and output (b) of chickpea under different hydroponic and soil conditions: *p<0.05, ***p<0.001.

The same regularity was observed in corn. The protein content in corn grown in soil was 1.2 times higher than that of corn cultivated in AH and CH. However, corn grown in CH and AH surpassed soil-grown corn in protein output by 2.6–2.8 times (Fig. 3).

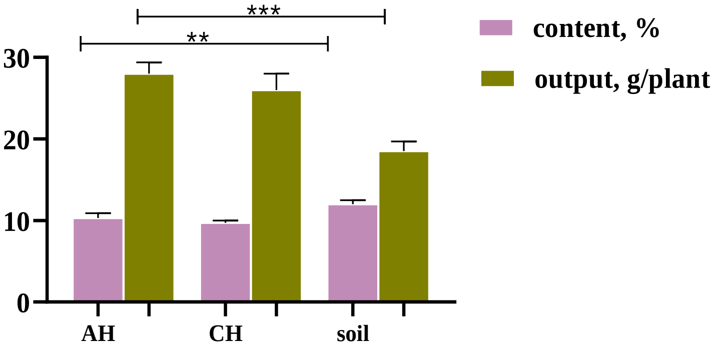


Figure 3. Protein content and output of corn in different hydroponics and soil conditions: **p<0.01, ***p<0.001.

The protein content in the studied grain crops fluctuated between 9.7 – 31.5% in CH and 11.7 – 24.0% in soil (Fig. 5a). Based on protein content, soil-grown crops followed the decreasing order: lentil - Flip 2007 3L > soybean > lentil - Talini 6 > chickpea - Flip 07-44B > chickpea - Karin > corn, while hydroponic-grown crops followed a slightly different ranking: soybean > lentil - Flip 2007 3L > lentil - Talini 6 > chickpea - Flip 07-44B > chickpea - Karin > corn. The protein output of the studied plants ranged from 12.2 – 34.0 g/plant in hydroponics and 2.5 – 33.6 g/plant in soil (Fig. 5b). Regardless of the cultivation method, the ranking of protein output remained consistent in both hydroponics and soil: chickpea (Flip 07-44B) > chickpea (Karin) > corn > lentil (Flip 2007 3L) > lentil (Talini 6) > soybean. Across all cultivation methods and zones, the lentil variety Flip 07-44B outperformed Talini 6 in protein content and output (Fig. 4, 5a, b).

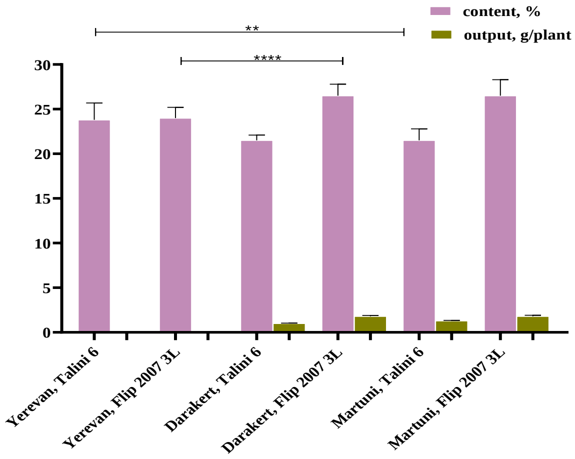


Figure 4. Protein content and lentil output in different soil conditions: **p<0.01, ****p<0.0001.

For Talini 6, the highest recorded protein content (23.8%) was in Yerevan, which was 1.1 times higher than in plants grown in Martuni and Darakert. In contrast, for Flip 2007 3L, the highest protein content was observed in Darakert and Martuni, exceeding that of Yerevan-grown plants by 1.1 times (Fig. 4).

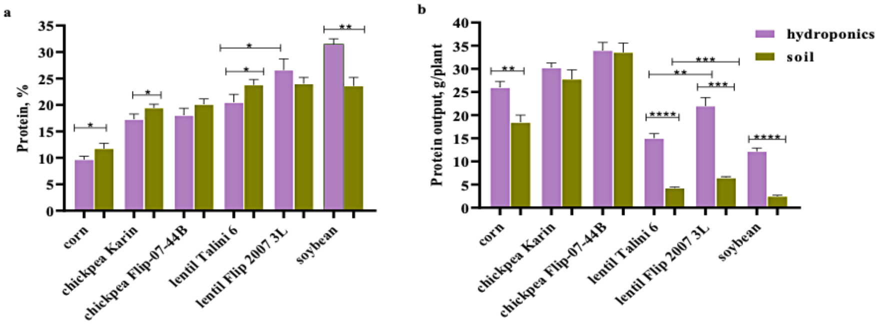


Figure 5. Protein content (a) and output (b) of grain crops in CH and soil conditions: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

In both hydroponic and soil cultivation, the output of BC in soybean followed this ascending order: LNA < PA < TSFA < LA < main FA**.** Compared to soil-grown soybean, the output of LNA, PA, TSFA, LA, and main FA was 2.4, 2.0, 2.0, 2.1, and 1.9 times higher in CH, respectively (Fig. 6).

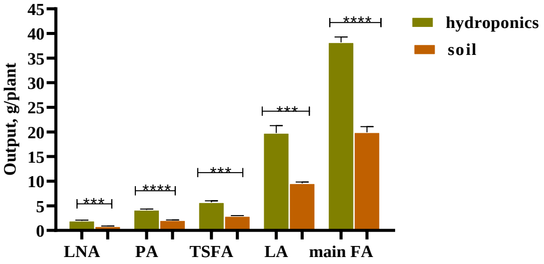


Figure 6. The output BC of soybeans in CH and soil conditions: ***p<0.001, ****p<0.0001.

Soil-grown *E. purpurea* plants were 1.6 – 1.8 times higher in polysaccharide content, 1.1 – 1.4 times higher in extract content and 1.3 – 1.4 times higher in flavonoid content than plants grown in CH and AH (Fig. 7a). However, in CH, *E. purpurea* exhibited 1.3 times higher output of extractive substances, 2.2 times higher phenylpropanoid output, and 1.1 times higher flavonoid output compared to soil-grown plants. In contrast, in AH, the production of polysaccharides, extractive substances, phenylpropanoids, and flavonoids was 2.1, 1.6, 1.7, and 1.6 times lower than in soil-grown plants (Fig. 7b).

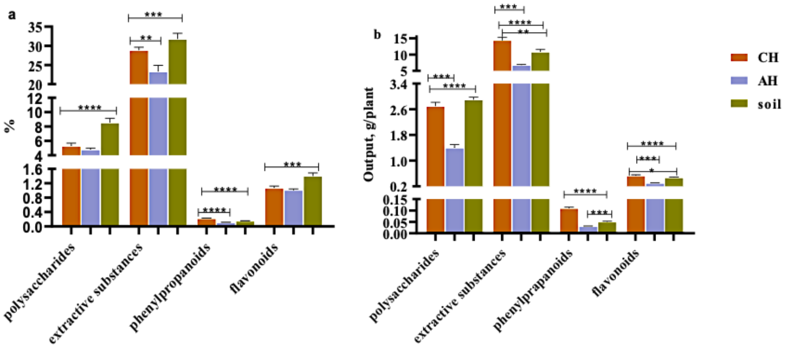


Figure 7. BC content (a) and output (b) of *E. purpurea* under different hydroponic systems and soil conditions: *p<0.05, ** p<0.01, ***p<0.001, ****p<0.0001.

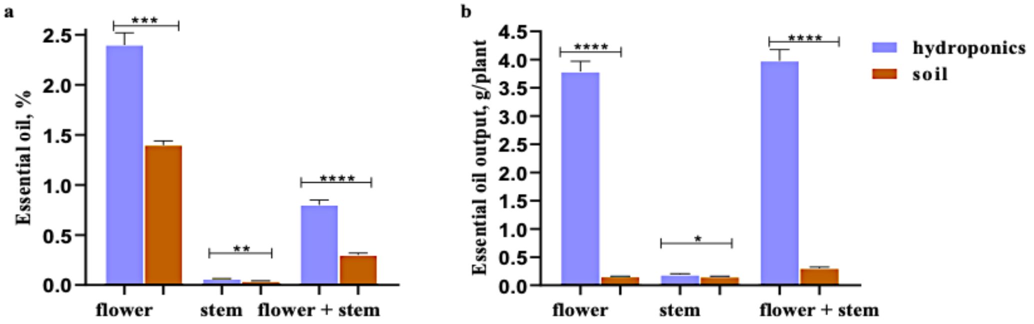


Figure 8. Essential oil content (a) and output (b) of *L. angustifolia* in CH and soil conditions: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

The essential oil content and output in *L. angustifolia* grown in CH were 1.7 times and 25 times higher, respectively, compared to plants cultivated in soil (Fig. 8a, b).

Figures 9 – 10 present a comparative analysis of gross β-radioactivity in grain crops and medicinal plants cultivated in different hydroponic systems and soil. The gross β-radioactivity of the edible parts of grain crops fluctuated between 70 – 400 Bq/kg in CH and 260 – 370 Bq/kg in soil, while medicinal plants exhibited values ranging from 400 – 550 Bq/kg in CH and 260 – 370 Bq/kg in soil. E. purpurea had higher gross β-radioactivity in CH than *L. angustifolia*, whereas the opposite was observed in soil-grown plants. Grain crops in CH were ranked in ascending order based on their gross β-radioactivity as follows: soybean > chickpea (Anush) > chickpea (Karin) > chickpea (Flip 07-44B) > lentil (Talini 6) > lentil (Flip 2007 3L) > corn. Conversely, soil-grown crops followed a descending order: soybean > lentil (Talini 6) > lentil (Flip 2007 3L) > chickpea (Flip 07-44B) > chickpea (Anush) > chickpea (Karin) > corn (Fig. 9, 10b). The gross β-radioactivity of CH-grown grain crops and medicinal plants was 1.1 – 2.2 times and 1.1 – 2.1 times higher, respectively, compared to those grown in soil (Fig. 9).

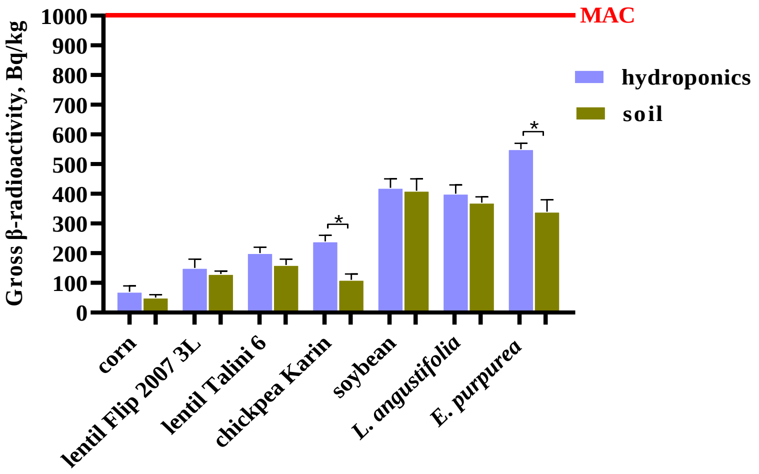


Figure 9. Gross β-radioactivity of grain crops and medicinal plants in CH and soil conditions: *p<0.05.

Under various growth conditions, the gross β-radioactivity of chickpea Anush and Karin varieties followed a decreasing range: DH > WSH > CH > soil (Fig. 10b). Additionally, chickpea cultivated in soil exhibited 2.0 – 4.4 times lower β-radioactivity compared to chickpea grown in DH, 1.1 – 4.0 times lower in WSH, and

1.2 – 2.2 times lower in CH. The chickpea Flip 07-44B variety had lower gross β-radioactivity than Anush and Karin in CH (1.1 times lower for both), in WSH (2.4 and 1.6 times lower, respectively), and in DH (1.5 and 1.2 times lower, respectively). However, Flip 07-44B exhibited 1.5 and 1.6 times higher β-radioactivity in soil compared to Anush and Karin, respectively. The lentil Talini 6 variety showed 1.3 times higher β-radioactivity than Flip 2007 3L in CH and 1.2 times higher in soil (Fig. 9). In both CH and soil, soybean exhibited 5.7 times and 8.2 times higher gross β-radioactivity than corn, respectively. Compared to lentil, soybean had 2.0-2.7 times higher β-radioactivity in CH and 2.6-3.1 times higher in soil. Similarly, soybean surpassed chickpea in β-radioactivity by 1.7 times in CH and 3.7 times in soil. According to the β-radioactivity, the different growth methods of *E. purpurea* followed a decreasing range: CH > AH > soil, whereas for corn, the order was AH > CH > soil (Fig. 10a).

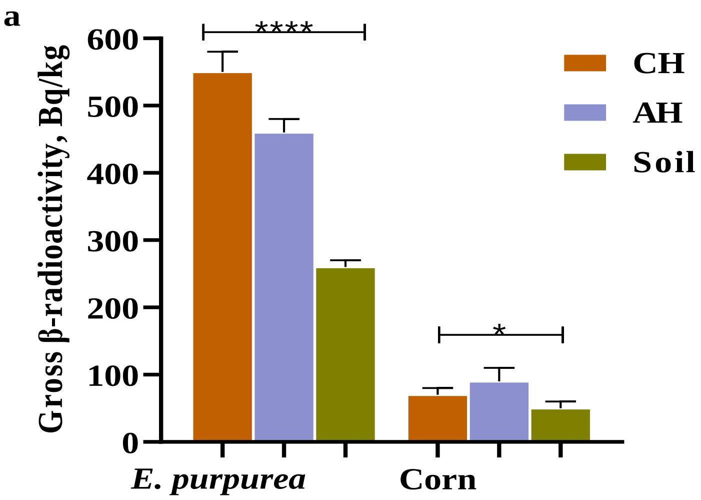


Figure 10. Gross β-radioactivity of *E. purpurea,* corn (a), and chickpea (b) under different hydroponic systems and soil conditions: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

DISCUSSION

Our research in the Ararat Valley has demonstrated that modifying plant cultivation conditions allows for regulating plant raw material composition based on its intended use. Additionally, we have found that abiotic and biotic factors—including cultivation methods, various hydroponic systems, plant species and varieties, and cultivation regions—significantly influence the content and output of BC, such as proteins, polysaccharides, flavonoids, phenylpropanoids, essential oil, TCFA, main FA, LA, LNA, and PA, as well as β-emitting RN in grain crops and medicinal plants. As a result, the radio-safe plant raw materials obtained, with their high BC content, present a promising potential as ingredients for functional foods and medicines. The biosynthesis of major secondary metabolites in plants occurs more intensively under hydroponic conditions. In hydroponics, soybeans exhibit higher LNA, PA, TSFA, LA, and main FA levels, while *L. angustifolia* produces more essential oil, and *E. purpurea* synthesizes higher amounts of phenylpropanoids. However, there are exceptions. The biosynthesis of protein in chickpea and corn and polysaccharides, extractive substances, and flavonoids in E. purpurea was more pronounced in soil-grown plants. Notably, due to their higher yield, hydroponically grown plants outperformed soil-grown plants in BC output. The exception was the release of polysaccharides in *E. purpurea*, which was greater in soil-grown plants.

In identical natural climatic conditions and within a zone of radioecologically equivalent intensity, plants exhibited varying levels of β-emitting RN. This variation is primarily influenced by the ability of different plant species to absorb mineral nutrients selectively and by their biological characteristics, including mineral nutritional properties, growth duration, anatomical structure, leaf shape, and size [3, 5, 36-38]. According to our data, grain plants such as corn, lentil, and chickpea demonstrate low levels of gross β-radioactivity. This aligns with previous findings on peas and fava beans, which had lower β-radioactivity than certain leafy vegetables, such as Chinese cabbage, lettuce, and tatsoi. Therefore, regarding radiological safety, grain crops are safer than leafy vegetables, making them a safer functional food ingredient. While hydroponic plants exhibited higher gross β-radioactivity than soil-grown plants, they contained lower levels of the most hazardous technogenic RN (^{137}Cs and ^{90}Sr), compared to soil-grown plants, by 1.1-1.8 and 1.1-2.1 times, respectively [36]. This may be attributed to the significantly lower radioactivity of ^{90}Sr and ^{137}Cs in hydroponic nutrient solutions than in soil [37]. Therefore, from a radiological safety standpoint, hydroponic plants are preferable to soil-grown plants as functional food ingredients.

CONCLUSION

By optimizing factors such as plant species and varieties, cultivation methods, radioecological zones, and hydroponic system types, it is possible to obtain high-quality, BC-rich, radio-safe plant material. At the IHP experimental station in the Ararat Valley, various grain crops (lentil, chickpea, corn, and soybean) and medicinal plants (*E. purpurea* and *L. angustifolia*) were cultivated using different hydroponic systems (CH, AH, WSH, DH) and the soil method. These plants are rich in BC and can be used as a healthy food or in medicine, depending on the purpose. Hydroponically grown plants demonstrated superior output of proteins, extractive substances, flavonoids, phenylpropanoids, essential oil, TCFA, main FA, LA, LNA and PA, compared to their soil-grown counterparts – highlighting hydroponics as a more efficient cultivation method. Additionally, hydroponic cultivation contributes to enhanced radio-safety, producing plant materials with lower radioactivity levels. Importantly, all plant material obtained, regardless of plant species, varieties, cultivation method and zone, were confirmed as radio-safe, with gross β-radioactivity levels meeting WHO radio-ecological safety standards (<1.0 Bq/g).

List of Abbreviations: AH, aquaponics; ANPP, Armenian Nuclear Power Plant; BC, bioactive compoundes**;** COVID-19, coronavirus disease; CH, classical hydroponics; CVD, cardio vascular disease; DH, drip hydroponics; *E.*, *Echinacea*; EC, electrical conductivity; FA, fatty acids; ICARDA, International Center for Agricultural Research in the Dry Areas; IHP, Institute of Hydroponics Problems; *L.*, *Lavandula*; LA, linoleic acid; LNA, linolenic acid; MAC, Maximum Allowed Concentration; PA, palmitic acid; RN, radionuclides; RF, Russian Federation; SARS, severe acute respiratory syndrome; TSFA, total saturated fatty acids; WHO, World Health Organization; WSH, water-stream hydroponics.

Authors' contributions: All authors contributed to this study.

Competing interests: The authors declare no conflict of interest.

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