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

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ABSTRACT

Background: It is known that various natural (^{40}K, ^{234}Th, ^{231}Th, etc.) and technogenic (^{90}Sr, ^{89}Sr, ^{137}Cs, ^{134}Cs, etc.) radionuclides (RN) may enter into the biosphere during the operation of nuclear power plants (NPP). Moving along the transfer chains of irrigation water – soil - plant and hydroponic nutrient solution – substrate - plant, the RN may penetrate human organisms and potentially cause various dangerous diseases. Thus, monitoring the gross β-radioactivity of plant materials (such as vegetables, medicinal plants, fruits, etc.) and ensuring the production of radioecologically safe plant raw materials is a contemporary challenge.Since 1996, we have been conducting radiomonitoring studies in Ararat Valley in the water-soil-plant ecosystem of the zones within the 2-15, 20, and 30 km radius of Armenian NPP (ANPP) technogenic influence.

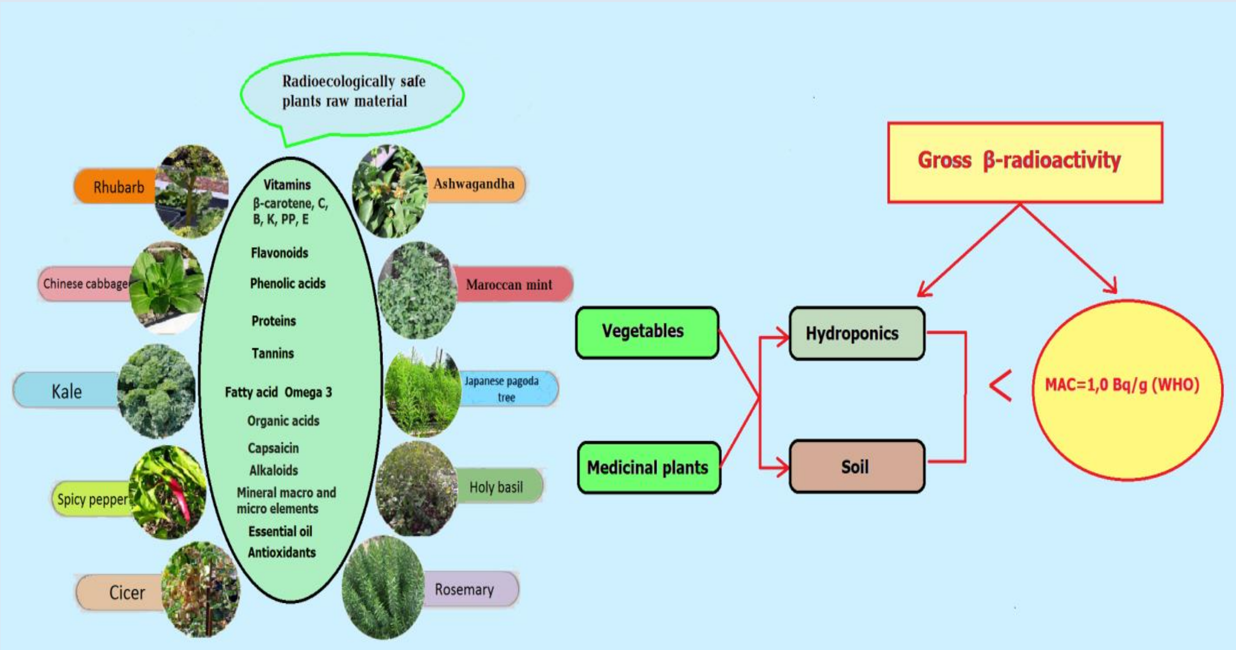
Objective: To reveal the optimal conditions for radio-ecologically safe plant raw material, we compared the gross β-radioactivity of the edible parts of several valuable vegetables (rhubarb - *Rheum undulatum* L., Chinese cabbage - *Brassica rapa subsp. Chinensis*, kale - *Brassica oleracea var. Sabellica* L., spicy pepper - *Capsicum frutescens* L., (varieties - “Bishops crown”, “Cayenne”, “Chili De arbol”, “Little Elf”, ”Fresno”), cicer - *Cicer arietinum* L., (varieties - “Kapuli” introduced from the USA, “Desi” introduced from Canada and “Leningradskaya 313” introduced from Russia)) as well as the plant raw materials of medicinal plants (ashwagandha - *Withania somnifera* L., Moroccan mint - Mentha spicatavar. Crispa, Marokko, Japanese pagoda tree - *Sophora japonica* L., holy basil - *Ocimum* *tenuiflorum L., and rosemary - Rosmarinus officinalis L.)*. These plants were introduced into Armenia and grown under various outdoor hydroponic systems (organic, NFT, water stream, drip, and classical hydroponics) and in soil.hydroponics) and soil. The studies were done at the Institute of Hydroponics Problems (IHP) in Ararat Valley (a zone of ANPP with a 30 km radius). It has concrete practical significance since it allows radio-ecologically safe plant raw material to be received.

Methods: The gross β-radioactivity of the plant samples was determined using a small background UMF-1500 radiometer.

Results: The results of the radiochemical study have shown that in the same zone of radio voltage and climatic conditions of Ararat Valley, several varieties of crops cultivated in hydroponic and soil conditions have accumulated different amounts of β-emitting technogenic and natural RN. The gross β-radioactivity of vegetables fluctuated between 230 - 590 Bq/kg in classic hydroponics and 140 - 480 Bq/kg in soil, and the gross β-radioactivity of medicinal plants was between 320 - 680 Bq/kg in hydroponics and 240 - 450 Bq/kg in soil. In both hydroponic systems and soil, the gross β-radioactivity of the edible parts of vegetables and medicinal plants follows the same decreasing order: for vegetables, the order is rhubarb > Chinese cabbage > kale > spicy pepper > chickpea; and for medicinal plants, the order is ashwagandha > Moroccan mint > holy basil > Japanese pagoda tree > rosemary.

Conclusion: Regardless of the crop varieties and cultivation methods (organic, water stream, drop, classical hydroponics, and soil culture), the plant raw material is considered radio-ecologically safe because their gross β-radioactivity doesn't exceed the radio-ecological safety threshold stated by the World Health Organization – 1000 Bq/kg.

Keywords: vegetables, medicinal herb, radionuclides, nutrient solution, radio-ecological safety



Gross beta-radioactivity of some vegetables and medicinal plants in conditions of outdoor hydroponics and soil culture in Ararat Valley

INTRODUCTION

According to the Power Reactor Information System (PRIS), there are 442 nuclear reactors working in 33 countries around the world. During the operation of Nuclear Power Plants (NPP), many natural (^{40}K, ^{228}Ra ^{234}Th, etc.) and technogenic (^{90}Sr, ^{89}Sr, ^{137}Cs, etc.) radionuclides (RNs) may enter the biosphere and spread around nuclear power plants (NPPs) within a radius of several tens of kilometers. RNs are the source of gross β-radioactivity. In the biosphere, radionuclides (RNs) can enter human organisms and cause dangerous diseases through the pathway of 'irrigation water – soil – plant.' The created situation requires control over the level of gross β-radioactivity of vegetable and medicinal plants used by humans, which must comply with the radioecological safety norm accepted by the World Health Organization (WHO) - 1.0 Bq/g. In this regard, controlling the level of gross β-radioactivity in vegetable and medicinal plant raw materials used by humans as functional foods and obtaining radio-safe plant raw materials is considered apriority issue [1-5].

Soilless culture of plants or hydroponics produces high-quality plant raw materials and increases plant bioactivity and efficiency. Hydroponics has the potential to reduce the entry of radionuclides (RNs) into the human body by allowing precise control over the chemical composition of plant raw materials [6-7]. Vegetables and medicinal plants are a rich source of bioactive compounds (BAC) (including vitamins, carotenoids, proteins, carbohydrates, organic acids, flavonoids, tannins, alkaloids, essential oil, phenolic acids, extractive substances, fatty acids, mineral macro- and microelements) Medicinal plants have been used in Armenia since time immemorial. According to the WHO, more than 3.5 billion people worldwide use plant-origin medicines. This is because medicinal plants are harmless, slightly toxic, have few side effects, and are cost-effective [8-21].

Here we studied several medicinal plants (ashwagandha (*Withania (W.) somnifera* L.), sophora (*Sophora (S.) japonica* L.), holy basil (*Ocimum (O.)tenuiflorum* L.), Moroccan mint (*Mentha (M.) spicata var. crispa, Moroccan*) and rosemary - *Rosmarinus (R.) officinalis* L.) and vegetables with medicinal properties (rhubarb (*Rheum (R.) undulatum* L.), spicy pepper (*Capsicum* (C.) *frutescens* L.), cicer (*Cicer (C.) arietinum* L.), kale (*Brassica (B.) var. Sabellica* L.) and Chinese cabbage (*Brassica (B.) rapa subsp. Chinensis*)) all of which were introduced into Armenia. They all have antioxidants, anti-inflammatory properties, and most have antitumor activity as well.

C. frutescens L. has antimicrobial, anti-obesity, and cardiovascular protecting effects [8-9]. *B. rapa subsp. Chinensis* is used in the treatment of cardiovascular and gastrointestinal diseases, anemia, burns, and vascular atherosclerosis [10]. *B. var. Sabellica* L. can replace meat products due to the high protein content. It is equal to fish products because of omega-3 fatty acid content. Kale protects the human body from diabetes, cardiovascular, gastrointestinal, and eye diseases, promotes normal brain development and regulation of the nervous system, and is helpful during infectious diseases, joint damage, allergies, and asthma [11-12].

C. arietinum L. is also a rich source of protein. It is aphrodisiac and has antidiabetic, antidiarrheal, estrogenic, hypocholesterolemic, anticonvulsant, hepatoprotective, and diuretic effects [13].

R. undulatum L. can be a rich source of vitamins C and K. It is often used in sweet dishes and helps digestion because of its high fiber content [14].

W. somnifera L. and *O. tenuiflorum* L. are adaptogenic medicinal plants. Ashwagandha has been used in traditional Indian medicine for over 3,000 years [15-16]. Holy basil is used in Ayurveda and traditional medicine in several Southeast Asian countries. It is used for the treatment of infectious and viral diseases, skin and liver diseases, pneumonia, cardiovascular diseases, asthma, atherosclerosis, and diabetes [17]. *M. spicatavar. crispa, Moroccan mint* has an antidepressant effect and is used to treat diabetes, asthma, bronchitis, lung, kidney, and skin diseases [18].

S. japonica L. is known as a source of the flavonol rutin, which belongs to the vitamin P group. Its preparations are used for the treatment of duodenal diseases, hypertension, diabetes, pneumonia, varicose veins, endarteritis, trophic ulcers, psoriasis, eczema, scurvy, and rheumatism [19].

R. officinalis L. is a spice plant with antimicrobial, antiseptic, neuroprotective, antibacterial, and antimutagenic properties [20-21].

Given the above considerations, the priority task is to determine the optimal conditions for producing the most radio-safe and high-quality plant material. This includes selecting plant varieties, growing methods, substrates, and the amounts of natural manure used in organic hydroponics (OH).. For this purpose, the comparative characteristics of the gross β-radioactivity and BAC of above-mentioned plants were studied under the conditions of various outdoor hydroponic systems (classical (CH), OH, nutrient film method (NFT), water-stream (WSH), drop (DH)) and soil culture in Ararat Valley. These studies have both scientific and practical significance.

MATERIALS AND METHODS

The studies, conducted from 2019 to 2023 in the territory of IHP located in the Ararat Valley (Yerevan, 30 km radius zone of Armenian NPP) fell under a comprehensive range of different outdoor hydroponic systems and soil conditions. The Ararat Valley, located at an altitude of about 850-900 m above sea level, has a very dry climate: the average annual temperature is 11.0-11.8 ^{0}C, the relative humidity is 40%, and the average annual precipitation is 200-300 mm [22].

In OH, manure mixed with the substrate was spread evenly on a polyethylene insulating film on the soil layer (800 g, 1600 g, 2400 g, 3200 g, or 4000 g of manure was used on 0.25 m^2 area). Plants were watered with artesian water as a spray from above. During the day, plants were watered 8-10 times.

In hydroponics, plants were nourished with the nutrient solution offered by Davtyan [22]. Artesian water was used for the preparation of the nutrient solution. Hydroponic substrates gravel, volcanic slag, and their mixture (with particles of 3-15 mm in diameter) were disinfected with a 0.05% solution of $KMnO_4$ before the planting. In WSH, the nutrient solution was pumped into the rizomedium of each plant 6-20 times a day for 10-15 seconds. In CH, plants were nourished 1-3 times a day, and in soil culture, plants were watered with artesian water once every 3-4 days. The soil was rich in P and K and had 1.5-2.5% humus. The agrotechnical rules were followed in the soil culture.

Radio and Biochemical Measurements: Vitamin C content in fresh plant material was determined according to Yermakov [23]. β-carotene in dry plant material was measured according to Sapozhnikov [24], and extractive substances, tannins, total flavonoids, and phenolic acids - according to SP RF XIV [23-24]; the content of photosynthetic pigments - according to Wetstein [25]. The antiradical activity was assessed using the free radical method [24], the quantitative determination of phenolic compounds was done by the Folin-Ciocalteu method [23-24], and the content of essential oil in fresh plant material by the Ginsberg method [26]. Total protein was determined using Kjeldahl's method [27]. The gross β-radioactivity of plant raw material was determined by the radiochemical method using a small background UMF-1500 radiometer [22]. Phytochemical analyses were replicated 3 times.

Statistical Analysis: The statistical analyses were done using GraphPad Prism 8. $P<0.05$ was considered statistically significant. β-radioactivity results were compared with the Maximum Allowed Concentration (MAC) [1].

For the study, plant samples were taken from edible parts of vegetables *(R. undulatum* L., *C. arietinum* L. (varieties: "Kapuli" introduced from the USA, "Desi" introduced from Canada and "Leningradskaya 313" introduced from Russia), *C. frutescens* L. (varieties: "Bishops crown", "Cayenne", "Chili De arbol", "Little Elf", "Fresno"), *B. rapa subsp. Chinensis*, *B. oleracea var. Sabellica* L.) and from overground parts and roots of medicinal plants.

RESULTS

Our results showed that BAC biosynthesis in crops was more intense in hydroponics (Fig. 1-5). The vitamin C content in spicy pepper, kale, Chinese cabbage, and holy basil grown in CH was 1.2 times higher than in soil variants (Fig 1a, b). In the case of rhubarb (Fig 1a) and Moroccan mint (Fig 1b), soil plants outperformed hydroponic ones by 1.7-2.4 and 1.2 times, respectively.

In CH, the content of extractive substances in kale and Chinese cabbage exceeded their content in the same plants grown in soil by 1.2-1.3 times (Fig 2b), and for holy basil, the soil results exceeded CH ones by 1.3 times (Fig 2b). The content of phenolic acids in Chinese cabbage, sophora, and essential oil content in Moroccan mint in CH variants were higher 1.2-1.9 times (Fig 2a, 3b) and 1.5 times (Fig 5a), respectively, compared to soil ones.

The content of flavonoids quercetin, rutin, and tannins in the CH variant of sophora was 1.2, 1.3, and 1.8 times higher than the same indicators of the soil variant, respectively (Fig 2a). The protein content of hydroponic cicer was 1.2-1.6 times higher than that of soil-grown cicer (Fig 4b).

The carotenoid content in ashwagandha was 1.7-2.2 times lower in soil plants compared to those grown in CH (Fig 5b). Omega-3 fatty acid content in CH kale exceeded the same indicator of soil kale by 1.2 times (Fig 3a).

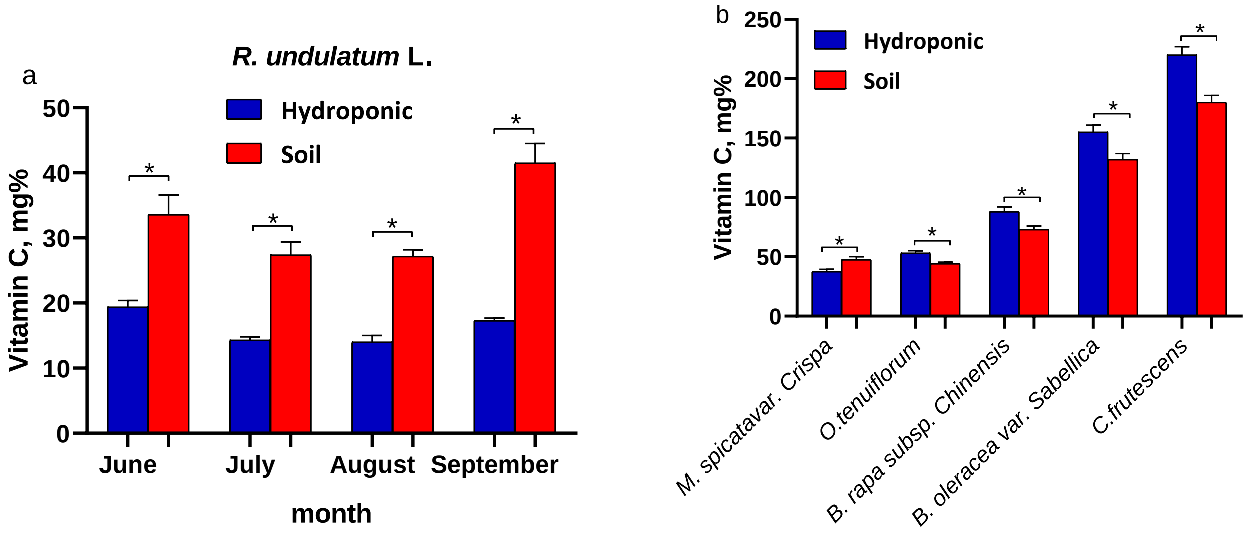


Figure 1: Vitamin C content in *R. undulatum* L. (a) and other plants (b) in CH and soil conditions: * - p<0.05

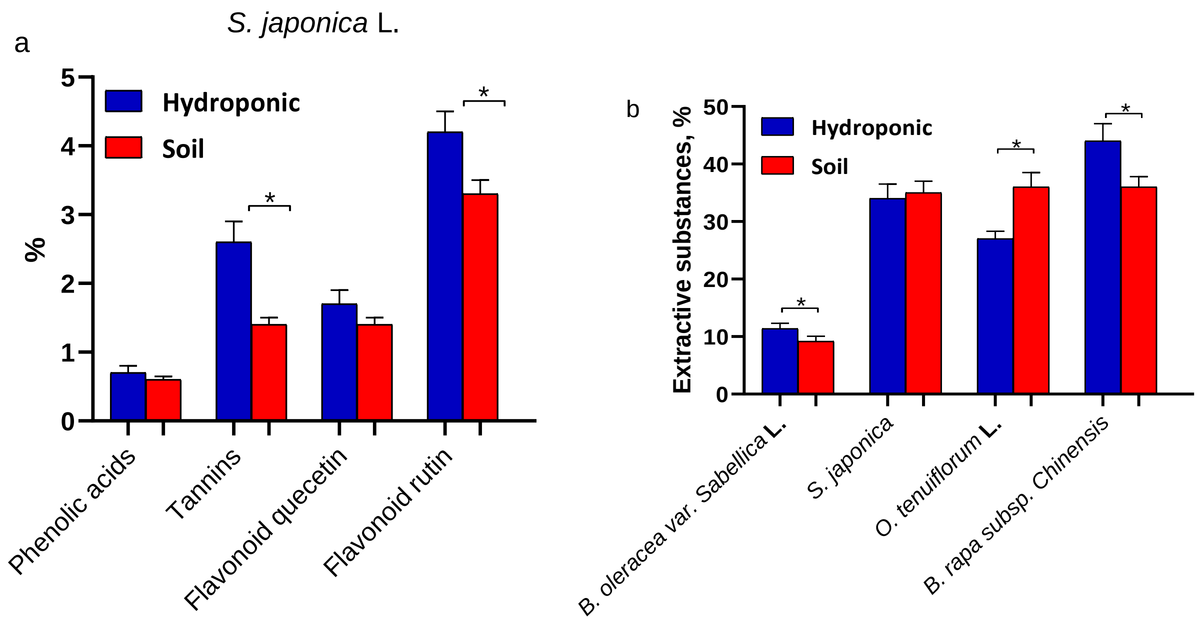


Figure 2: Biochemical indices of *S. japonica* (a) and other plants (b) in CH and soil conditions: * - p<0.05

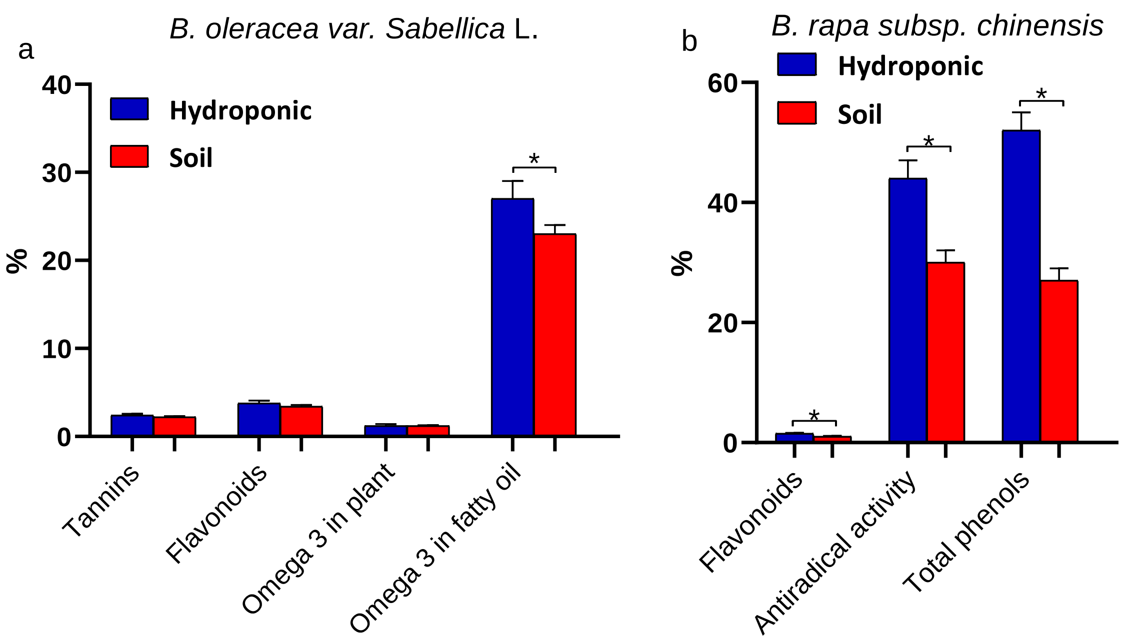


Figure 3: Biochemical indices of *B. oleracea var. Sabellica* L. (a), and *B. rapa subsp. Chinensis* (b) in CH and soil conditions: * - p<0.05

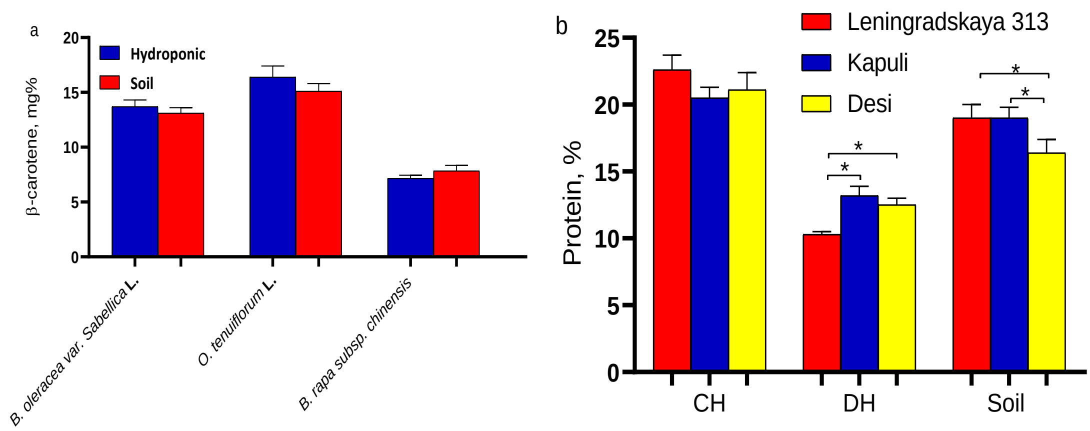


Figure 4: β-carotene content in *B. racea var. Sabellica* L., *O. tenuiflorum* L, and *B. rapa subsp. Chinensis* (a) and protein content in *C. arietinum* L. (b) in CH and soil conditions: * - p<0.05

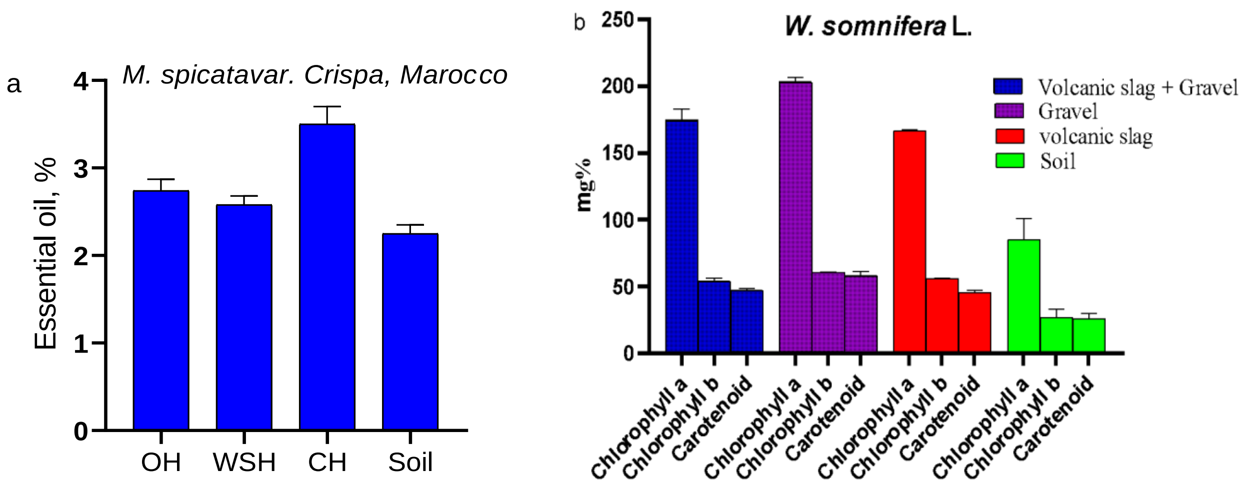
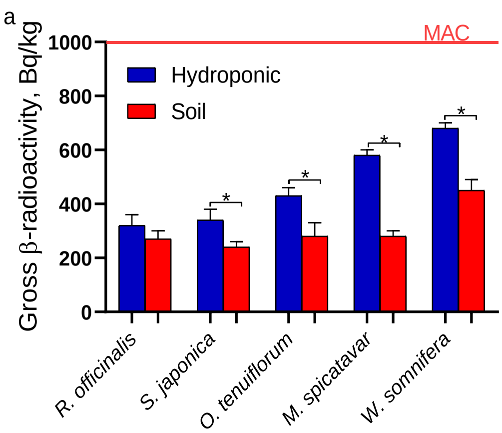


Figure 5: Essential oil content of *M. spicata var. crispa* Moroccan (a) and chlorophyll "a," "b" and carotenoid content of *W. somnifera* L. (b) in CH and soil conditions

It was established that different growing conditions in the same climatic zone and radioecological intensity of the Ararat Valley had a certain impact on the level of gross β-radioactivity of plant material (Fig. 6 a, b, Fig. 7 a, b, c, d, e, f). The gross β-radioactivity of edible parts of vegetables ranged from 230 to 590 Bq/kg in CH and from 140 to 480 Bq/kg in soil. For medicinal plants, the β-radioactivity ranged from 320 to 680 Bq/kg in CH and 240 Bq/kg in soil. The gross β-radioactivity of vegetable plants grown in CH was 1.2-1.8 times higher than in soil plants. Regarding medicinal plants, CH plants' gross β-radioactivity prevailed over that of soil ones 1.2-2.1 times. According to the gross β-radioactivity, medicinal plants and vegetables form the same descending series in CH and soil: ashwagandha > Moroccan mint > holy basil > Japanese sophora > rosemary (Fig 6a), and rhubarb > Chinese cabbage > kale > pepper > cicer (Fig 6b), respectively. Cicer seeds are inferior to leaves in terms of gross β-radioactivity: in hydroponic systems by 1.1-4.0 times and in soil by 1.2-2.0 times. The most radio-safe cicer seeds were obtained from WSH and soil culture. Seeds of variety "Desi" are superior to the varieties "Kapuli" and "Leningradskaya 313" by the gross β-radioactivity 1.1-1.5 times in WSH and 1.2 times in soil. Cicer seeds were inferior to leafy vegetables, rhubarb, Chinese cabbage, and kale by gross β-radioactivity 1.4-2.6 times in CH and 2.1-3.4 times in soil (Fig 7a). In CH, the best radio-safe substrate for rhubarb cultivation was the gravel + volcanic slag mixture, where the plant's leaves and petioles are 1.1-1.4 times inferior to those of both gravel and volcanic slag plants by the β-radiating RN content. The petioles of rhubarb are more radio-safe than the leaves by RN content 1.1-1.2 times in hydroponic substrates and 1.5 times in soil (Fig 7c).

"Little Elf" and "Fresno" species were inferior 1.1-1.3 times to "Bishops crown", "Cayenne", and "Chili De Arbol" species by gross β-radioactivity (Fig 7d). According to the increase in the level of gross β-radioactivity of crops, studied hydroponic systems form the following series: OH >NFT >CH > WSH (Fig 7abe). By the RN content, Chinese cabbage and kale in WSH were inferior to those in OH (1.4 and 1.5 times) and CH (1.1 and 1.2 times) (Fig 7b). In the case of OH, with the increase of used manure from 800 g to 4000 g, it was recorded an increase of gross β-radioactivity of Moroccan mint from 430 to 800 Bq/kg (Fig 7e). Regardless of cultivation conditions, different organs of ashwagandha exhibit the following descending order in terms of gross β-radioactivity: leaf > seed > root.

Ashwagandha roots with gross β-radioactivity were 2.8-3.3 times inferior to leaves, 1.2-1.5 times to seeds in different substrates, and 4.1 times to leaves and 2.3 times to seeds in soil. In addition, the gravel + volcanic slag substrate provided the most radio-safe plant material for the ashwagandha root. In that variant, the gross β-radioactivity of ashwagandha roots was lower than that of the volcanic slag (1.2 times) and gravel (1.3 times) variants (Fig 7f).



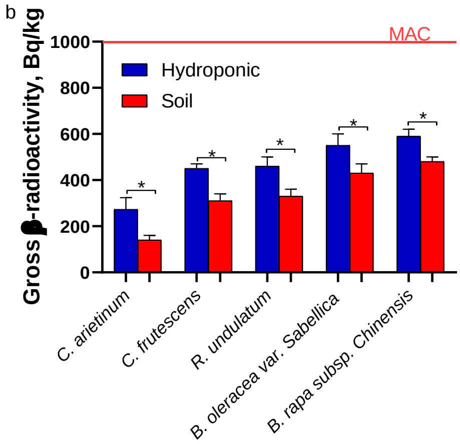


Figure 6: Gross β-radioactivity of medicinal (a) and vegetables plants (b), grown in different hydroponic and soil conditions: * - p<0.05

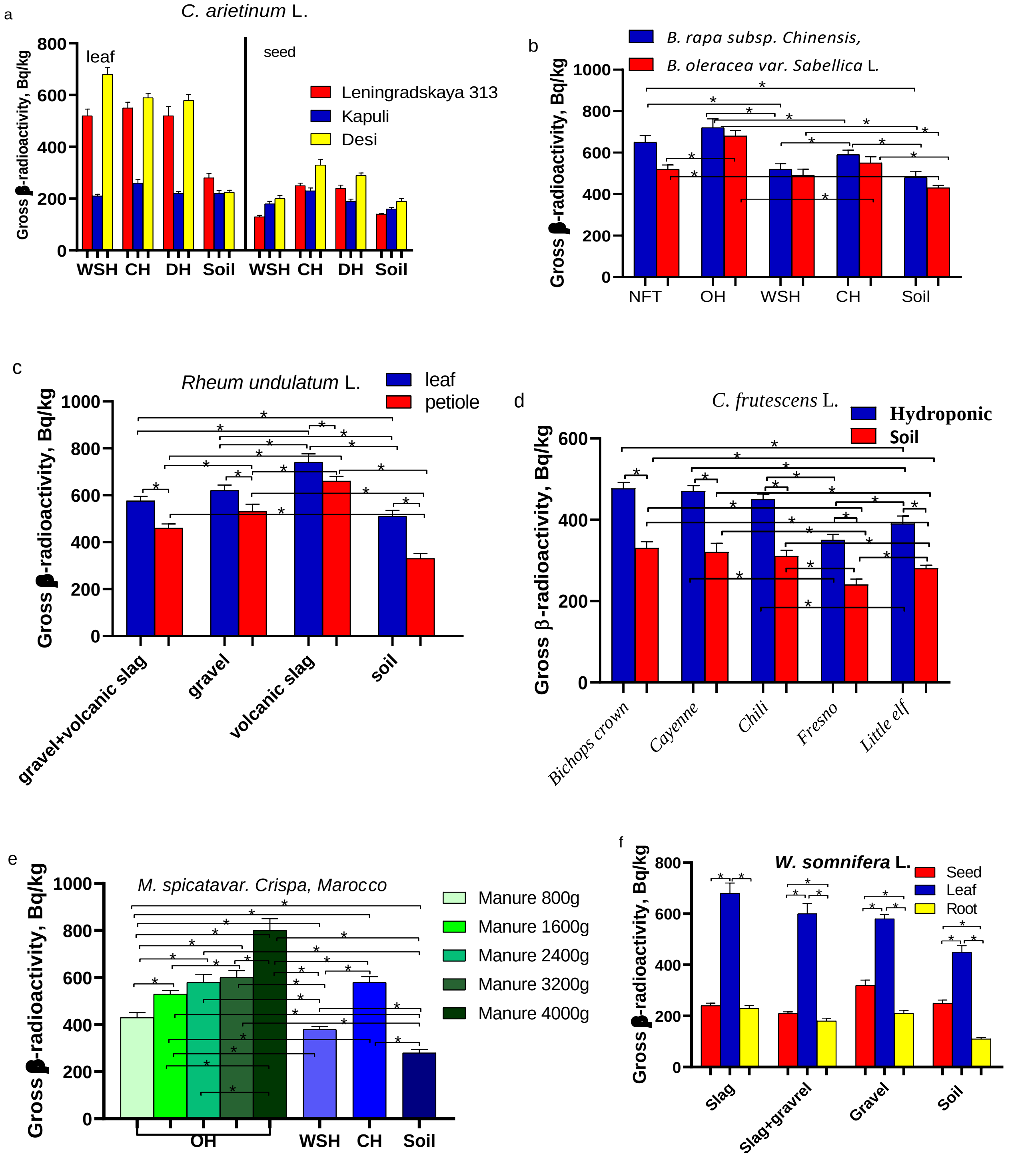


Figure 7: Gross β-radioactivity of vegetables (a,b,c,d) and medicinal plants (e,f) grown in different hydroponic and soil conditions: * - p<0.05

DISCUSSION

Our long-term studies have shown that plant growing conditions have a certain effect on the BAC and β-emitting RN content in vegetables and medicinal plants. In hydroponics, the optimal air-water-nutrient regime ensures high biological activity of crops, contributing to the increase of BAC content compared with soil crops. Exceptions were for rhubarb and Moroccan mint, in which the vitamin C biosynthesis was more intensive in the soil, and for holy basil, where the extractive compounds biosynthesis was higher in soil. The level of gross β-radioactivity of crops varies under different hydroponic and soil conditions. The biological characteristics of individual plant species (peculiarities of mineral nutrition, duration of vegetation, anatomical structure, shape, size of leaves, etc.) and the ability to selectively absorb mineral nutrients played a key determining role. The superiority of hydroponic crops over soil plants in gross β-radioactivity is explained by the fact that the amount of K (^{40}K) absorbed by plant roots from hydroponic nutrient solution is more than from soil. It is known that natural RN ^{40}K stands out with the greatest β-radioactivity among β-emitting RN, as a result of which the gross β-radioactivity of crops is mainly determined by the amount of K contained in plantsThe radionuclide ^{40}K constitutes 0.0119% of potassium and is a β-emitter 89.33% of the time and a γ-emitter 10.67% of the time [22]. Our studies have shown that the WSH method, which involves minimal nutrient use and automated, irreversible nutrient injection into the plant rhizomedium, results in more radio-safe plant material compared to OH, NFT, DH, and CH systems [22]. According to our studies, the share of the most dangerous controlled technogenic RN (^{90}Sr, ^{137}Cs) in the gross β-radioactivity of plants ranged between 2.3-6.8% in hydroponic plants and between 3.0-12.8% in soil plants. Thus, hydroponic plants can be considered more radio-safe than soil plants. This is probably explained by the relatively low content of ^{90}Sr and ^{137}Cs in the hydroponic nutrient solution compared to the soil [22].

Cicer is a radio-safe legume vegetable. Depending on the amount of manure used in OH (800-4000g), the increase in the gross β-radioactivity of Moroccan mint (1.9 times) is due to the presence of natural RN ^{232}Th, ^{226}Ra, and ^{40}K in the manure. According to the content of natural RN in manure, they are in the following decreasing order: $^{40}K > ^{226}Ra > ^{232}Th$ [22]. Therefore, exceeding 800g of manure in OH is not beneficial from a radio-ecological point of view.

CONCLUSION

The vegetables and medicinal plants under study, which were grown in Ararat Valley in both hydroponic and soil conditions, are rich in BAC and can be used for medicinal purposes and as high-quality, radio-safe functional foods. Regardless of the type of crops and the method of cultivation, the received plant material is radio-safe, as its gross β-radioactivity level corresponds to the WHO radio safety norm of 1.0 Bq/g. In terms of BAC content, cultivating crops in CH is more efficient than in soil. In terms of radio safety, growing crops in WSH is more beneficial than in CH, OH, NFT, DH, and soil.

A practical suggestion:

In terms of radio safety, it is beneficial:

- to apply gravel + volcanic slag substrate in case of cultivation of rhubarb and ashwagandha by CH method
- to use up to 800 g of manure in OH;
- to cultivate vegetables and medicinal plants by WSH method;
- to use "Fresno" and "Little Elf" varieties of spicy pepper and "Leningradskaya 313" and "Kapuli" varieties of cicer in food.

Abbreviations: BAC - biologically active compounds; CH - classical hydroponics, DH - drop hydroponics; OH - organic hydroponics; IHP – Institute of Hydroponics Problems; MAC - Maximum Allowed Concentration; NFT - nutrient film technique; PRIS - Power Reactor Information System; RN – radionuclides; SP - State Pharmacopoeia; WHO - World Health Organization; WSH - water-stream hydroponics.

Authors Contribution: All authors contributed to this study.

Competing interests: The authors declare no conflict of interest.

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