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SHAMILOV E. N.[1]✉, ABDULLAYEV A. S.[1], SHAMILLI V. E.[1], AZIZOV I. V.[2]

[1] Institute of Radiation Problems of the Ministry of Science and Education of the Republic of Azerbaijan, Azerbaijan Republic, AZ1143, Baku, B. Vahabzadeh, 9, ORCID: 0000-0002-5308-8718, 0000-0002-6338-4596

[2] Institute of Molecular Biology and Biotechnology of the Ministry of Science and Education of the Republic of Azerbaijan,
Azerbaijan Republic, AZ1073, Baku, Pr. Matbuat, 2A, ORCID: 0000-0002-5910-3923

✉ *elshanshamil@gmail.com*

STUDYING THE COMPOSITION AND ANTI-RADIATION PROPERTIES OF SAGE (*SALVIA OFFICINALIS* L.) IN WHEAT SEEDS

Aim. The purpose of the research was to study the chemical composition, develop methods for obtaining an extract from the leaves of *Salvia officinalis* L. and study their anti-radiation properties of irradiated wheat seeds of the Guneshli variety. ***Methods.*** Using qualitative reactions and chromatography, the content of lipids, essential oils, diterpene acids, phenolic compounds, macro- and microelements in plants was determined. Wheat seeds were irradiated using a URI installation (K-25) at a dose rate of 13.4 rad/sec, at a dose of 200 Gy. The amount of chlorophyll pigments, carotenoids, and malondialdehyde was measured using a spectrophotometer. Chlorophyll fluorescence in leaves was determined using a MINI-PAM device. ***Results.*** According to the results of morphological and physiological-biochemical parameters of seedlings, 0.01 % and 0.001 % *Salvia officinalis* extract has a positive effect on growth and development, increases the maximum quantum yield of PS II and reduces the yield of lipid peroxidation product. ***Conclusions.*** It has been established that *Salvia officinalis* extract has a radioprotective effect and can be used as a radioprotector for some agricultural plants.

Keywords: gamma radiation, malondialdehyde, photosynthetic pigments, growth and development, *Salvia officinalis*.

Sage is a well-known medicinal plant and is often used in medical practice around the world. In many countries, for example, England, USA, Germany, France, Poland, Austria, China, it is included in pharmacopoeias. The plant has many medicinal properties, has an antimutagenic effect, and protects the genetic apparatus from the damaging effects of carbon tetrachloride. By influencing nicotinic and muscarinic receptors in the brain, sage improves the processes of memorizing and retrieving information from memory. Experimental studies have shown that carnosol (salvin) from sage can serve as a therapeutic agent for multiple sclerosis and encephalomyelitis. Rosmarinic acid, which is found in sage leaves, has anxiolytic-like effects. Plant extracts have nootropic properties, due to their antioxidant properties, and have an antitumor effect. It has been established that the antioxidant effect of sage extract in food products containing fats and vitamins is associated primarily with carnosolic acid and its derivatives. Sage essential oil has a detrimental effect on cancer cells of the oral mucosa and cells of other types of tumors. Extracts of medicinal sage inhibit the process of angiogenesis, thereby having an antimetastatic effect. Manool, a diterpene isolated from sage leaves, selectively has a detrimental effect on tumor cells without affecting normal cells [1, 2, 3].

The flora of Azerbaijan is rich and diverse. Currently, it is represented by 4,745 species belonging to 193 families and 900 genera. Among them are many useful plants, including medicinal ones, which are distributed throughout the republic – in forests, steppes, semi-deserts, deserts, as well as weeds in crops, gardens and vegetable gardens. 500 species of the genus Lamiaceae have been recorded throughout the world, of which 40 species are in the flora of the Caucasus, and 27 species in the flora of Azerbaijan. In Azerbaijan, 2 species of the sage genus are grown, the remaining species grow wild. The main species of the essential oil genus. Medicinal sage (*Salvia officinalis* L.) was accepted as an official medicinal plant in state pharmacology [4].

Due to its rich chemical composition, it is widely used in all areas of medicine as an antioxidant, immunomodulatory, and anti-inflammatory agent [5, 6].

Based on the above, it seems interesting to study the anti-radiation properties of the extract of medicinal sage (*S. officinalis*), growing in Azerbaijan.

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The purpose of our research was to study the chemical composition, develop methods for obtaining extracts from the leaves of *Salvia officinalis* and study their anti-radiation properties of irradiated wheat seeds of the Gunashli variety. In parallel, experiments are ongoing to study the radioprotective properties of the extract in small laboratory animals.

Material and methods

For the research, we used medicinal sage leaves in filter bags produced by Herba Flora, purchased from a retail pharmacy chain. Phytochemical studies were carried out to study the chemical composition. Using qualitative reactions and chromatography, the content of lipids, essential oils, diterpene acids, phenolic compounds, macro- and microelements in plants was determined.

Chloroform extraction was performed to obtain lipids. Gas-liquid chromatography was used to study fatty acids in lipids. Chromatogram conditions: column size 250 – 0.3 cm; stationary phase – chromatron; carrier gas is nitrogen, hydrogen and leak rate, nitrogen – 30 ml/min: injector temperature - 185°C, detector temperature -190°C. Palmitic, linolic, linolenic and oleic acids were found in the studied lipids [7].

To study the presence of carotenoids in lipids, thin layer chromatography was used. In daylight, carotenoids appeared in the form of their characteristic yellow or orange spots, and in UV light they appeared as a coffee color. Afterwards, the chromatogram was developed with a 10 % alcohol solution of phosphomolybdic acid and dried in an oven. Carotenoids appeared in the form of their characteristic blue spots. In the studied lipid samples, carotenoids were quantified using a spectrophotometric method.

Alcoholic extracts from sage leaves were prepared by re-extracting the crushed raw material with 70 % isopropyl alcohol by heating in a refluxing water bath for three hours. Extraction was carried out with a general ratio of raw materials and extractant of 1:20, with constant stirring with a magnetic stirrer.

The determination of the main groups of biologically active substances was carried out by thin layer chromatography: sorbent – silica gel SG 60 (Merck), mobile phase – toluene: ethyl acetate. The test solution was prepared in the following way: 15 ml of the extract was placed in a separatory funnel and 20 ml of pentane was removed three times. The combined extracts were dried with anhydrous sodium sulfate, filtered and evaporated to dryness in a water bath at $t \leq 50°C$, the residue was dissolved in 3 ml of toluene. When analyzing a model alcoholic extract of *Salvia officinalis*, typical chemical compounds eucalyptola, α-thujone, β-thujone, camphor, ledol, manool, β-caryophyllene, α-humulen were identified.

The second stage of our research was to study the anti-radiation activity of the extract on the growth and development of wheat seedlings from the "Guneshli" variety, irradiated at a dose of 200 Gray.

In experiments with plants, wheat seeds of the "Guneshli" variety were used. Wheat seeds were irradiated at a dose of 200 gray at the URI installation (K-25) at a dose rate of 13.4 rad/sec, source – 60°C. Experiments were carried out in plastic vessels measuring 10x20 cm in five repetitions. Morphological parameters and growth dynamics of wheat seedlings were measured every 7 days for a month.

In parallel with laboratory experiments, field experiments were also carried out. The amount of chlorophyll pigments, carotenoids, and malondialdehyde was measured using a Multiscan GO spectrophotometer. Chlorophyll was extracted from the leaves with 96 % ethyl alcohol. Chlorophyll a (665 nm), chlorophyll b (649 nm) and carotenoids (440 nm) were determined spectrophotometrically from their absorption spectra. The content of pigments was calculated based on the fresh weight of leaves (mg/g) using the Winterman method. Chlorophyll fluorescence in leaves was measured using a MINI-PAM instrument (Photosynthetic Yield Analyzer, Germany). Using the formulas $F_v = F_m - F_0$ and F_v/F_m, the efficiency of photochemical energy conversion in the second photosystem was determined.

To determine the MDA content, freshly collected plant leaves were homogenized with a 5 % solution of trichloroacetic acid and then centrifuged (12000 g) for 10 min at 27°C. Equal volumes of supernatant and 0.5 % thiobarbituric acid were then added to 20 % trichloroacetic acid and incubated at 96°C for 30 minutes and quickly cooled in an ice bath. After centrifugation at 12000 g for 10 min, the optical density of the superprecipitation liquid was determined at 532 and 600 nm. Data analysis and statistical analysis were performed using Microsoft Excel. Statistical analysis was carried out using the statistical package Stat Graphics Plus 5.1. Mean values were compared using Duncan's multiple test ($p=0.05$).

Results and discussion

During field experiments, the effect of 0.1 %, 0.01 % and 0.001 % percent solutions of the extract on the growth, development, and photosynthetic activity of seedlings obtained from irradiated seeds of the "Guneshli" wheat variety at a dose of 200 gray

was studied. Morphological, physiological and biochemical studies of wheat seedlings were carried out in 5 variants of four repetitions: control, irradiated and treated with solutions of *Salvia officinalis* extract in three concentrations (0.1 %; 0.01 %; 0.001 %). The studies were conducted at different stages of wheat seedling development to observe their changes over time. In these experiments, the best results in growth and development were shown by seedling seeds treated with 0.01% and 0.001 % solutions of *Salvia officinalis*.

Based on the results of morphological indicators, as indicated, we can conclude that the extract has a positive effect on the growth and development of wheat seedlings.

At the initial stages of development in the first 10 days, the effect was not noticeable. At the end of the first month, seedlings obtained from gamma-irradiated seeds and treated with a 0.01 % solution of *Salvia officinalis* showed better results in growth and development. At the final stage of the experiments, the length of the seedlings was equal to that of the intact variant and even exceeded them (fig. 1).

As is known, when free radicals formed subsequently by radiation interact with the lipids of cell membranes, lipid peroxidation occurs. One of the end products is malondialdehyde (MDA), which is an indicator of the degree of damage to an organ or the whole organism. To determine the amount of malondialdehyde, the thiobarbituric method was used [8] (fig. 2).

As can be seen from the graph, as a result of irradiation, the peroxidation product doubles, and percentage solutions of the extract reduce the MDA product by about 30 percent. This means that the extract reduces stress levels and promotes plant development.

Based on the results of the experiments, it can be said that *Salvia officinalis* extract also has a positive effect on the physiological and biochemical parameters of seedlings, reducing the yield of lipid peroxidation product.

It is known that the efficiency of the primary processes of photosynthesis is reflected in the appearance of plants. Therefore, to study the radioprotective properties of the extract, along with the study of morphological parameters, we also studied the effect of different concentrations of the extract on the yield of photosynthetic pigments and carotenoids in gamma-irradiated wheat seedlings. It has been proven that some plant extracts and their compositions, complexes of various biogenic metals, increasing the content of chlorophylls and activating antioxidant enzymes, lead to increased protective and adaptive reactions of plants [9, 10]. Below are the results of experiments studying the effect of *Salvia officinalis* extract on the amount of photosynthetic pigments – chlorophylls a and b and carotenoids.

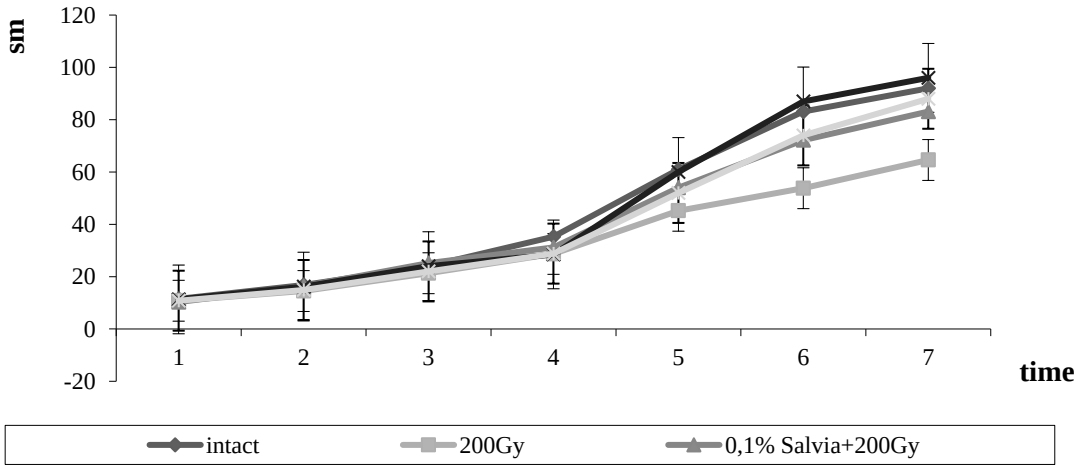

Fig. 1. Dynamics of growth of wheat seedlings treated with concentrations (0.1 %, 0.01 % and 0.001 %) of *Salvia officinalis* extract for comparison with control and irradiated variants. Statistically significant difference from control level ($p < 0.05$).

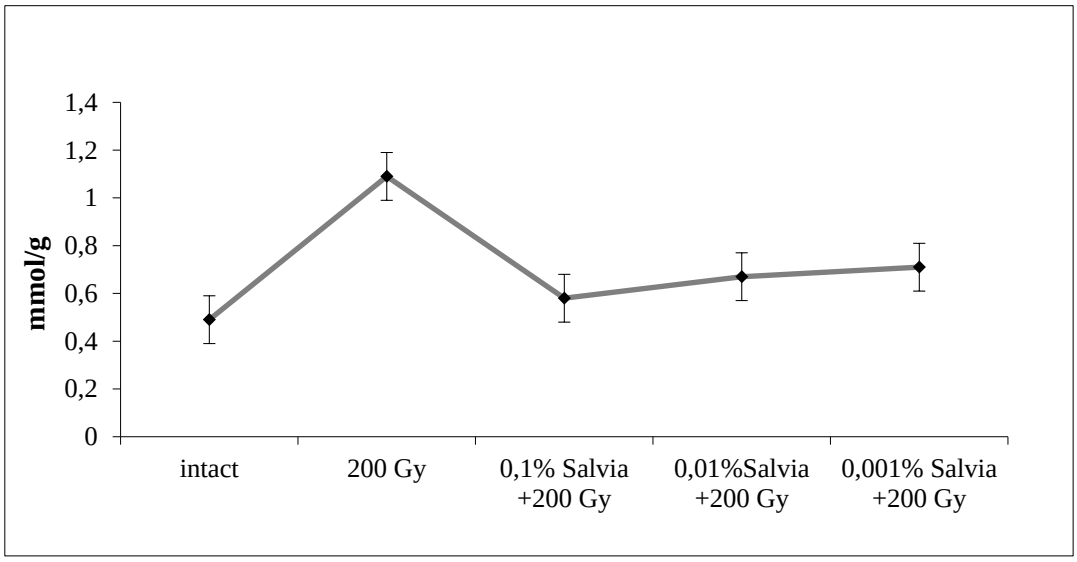

Fig. 2. The yield of malondialdehyde in wheat seedlings irradiated and treated with different concentrations of *Salvia officinalis* extract (mmol/g). Statistically significant difference from control level ($p < 0.05$).

As can be seen from fig. 3, in the irradiated variant there is a decrease in the amount of photosynthetic pigments – chlorophylls a and b and carotenoids, and in the variant with *Salvia officinalis* + 200 Gy, treated with a 0.01 % extract solution, there is a significant increase in the amount of photosynthetic pigments and carotenoids compared to the irradiated variant.

To determine the effect of *Salvia officinalis* extract on photosynthetic activity, fluorescent characteristics (maximum quantum yield of PS II – Y) in seedlings of gamma-irradiated wheat seeds were studied. Fluorescent methods are based on the fact that chlorophylls in photosynthetic membranes serve as a natural indicator of the state of plant cells. Under the influence of unfavorable conditions, including radiation, changes in chlorophyll fluorescence occur, which serve as a source of information.

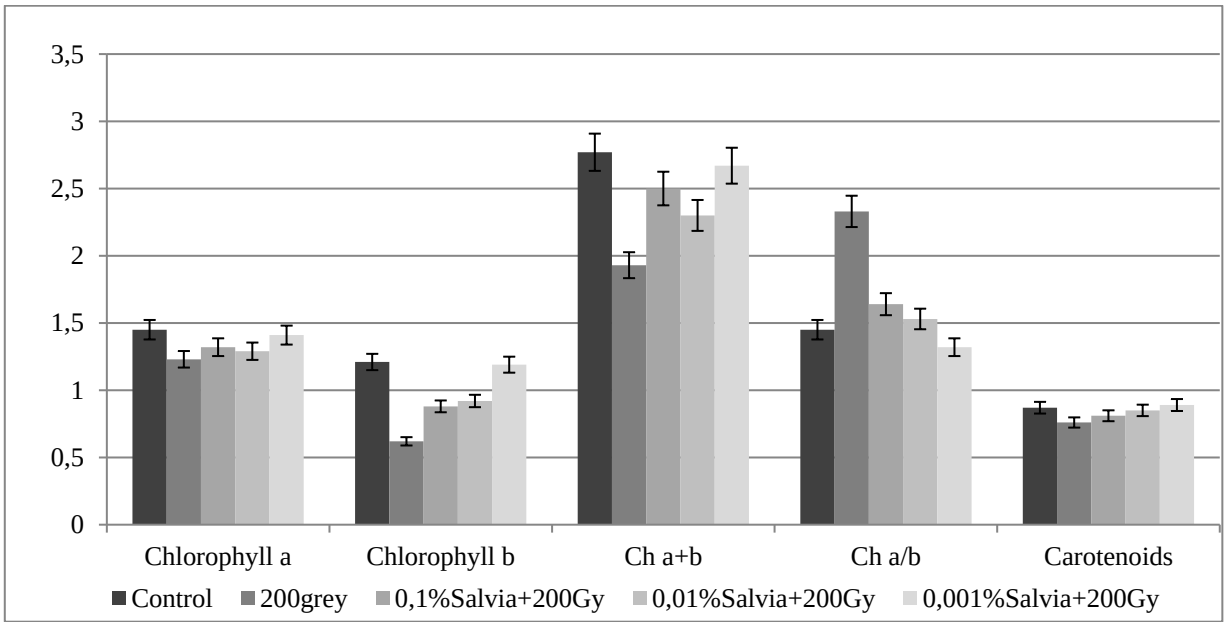

Fig. 3. Effect of different concentrations of *Salvia officinalis* extract on the amount of photosynthetic pigments chlorophylls a and b and carotenoids in irradiated wheat seedlings (mg/g). From left to right – control, irradiated variant (200 Gy), variant with 0.1 % solution of *Salvia officinalis* + 200 Gy, variant with 0.01 % solution of *Salvia officinalis* + 200 Gy, variant with 0.001 % solution of *Salvia officinalis* + 200 Gy. Statistically significant difference from control level ($p < 0.05$).

Disturbances in the primary processes of photosynthesis are reflected in changes in chlorophyll fluorescence. To assess the maximum efficiency of PS2 (photosystem II), the index of maximum quantum efficiency of PSII – F_v/F_m – is used. This indicator provides information about the potential quantum efficiency of PSII. A decrease in this value may indicate a stressed state of the plant and partial damage to PS II [11].

In previous studies, we discovered the radioprotective effect of phytoextracts and compositions of different extracts in wheat seedlings irradiated with different doses [12, 13]. The radioprotective effect of *Salvia officinalis* extract is similar to the effect of substances of natural origin that we studied.

The results of experiments studying photosynthetic activity showed that treatment of wheat seeds with a 0.01 % solution of *Salvia officinalis* extract before irradiation at a dose of 200 Gy increases the maximum quantum yield of PS II (fig. 4).

Conclusions

It has been established that a 0.01 % solution of *Salvia officinalis* extract eliminates the harmful effects of gamma radiation, normalizes plant growth, the synthesis of photosynthetic pigments in leaves, the functioning of photosystem II in chloroplasts and reduces the process of lipid peroxidation of cell membranes. The extract can be used as a radioprotector for some agricultural plants.

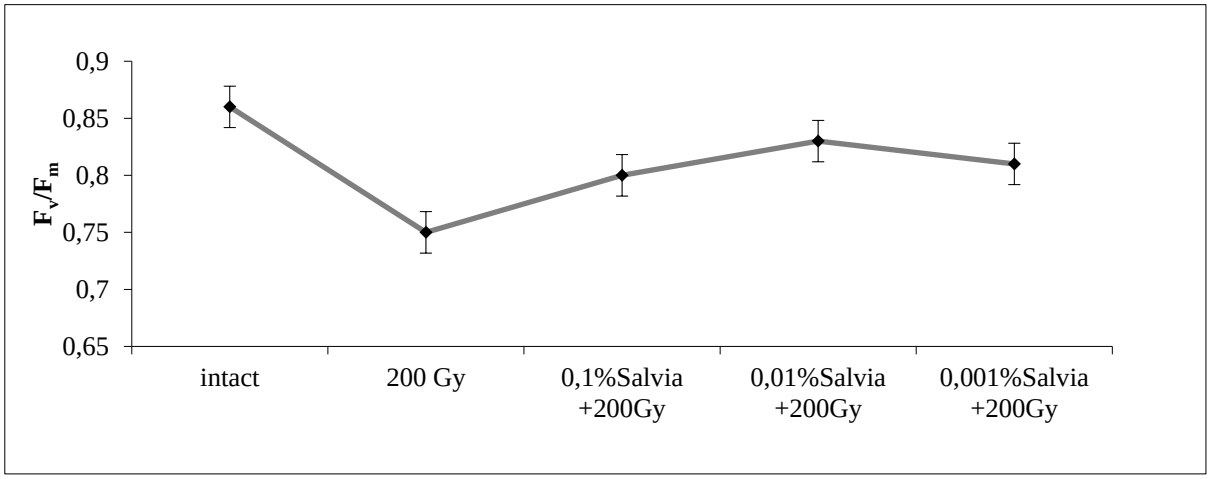

Fig. 4. The influence of different concentrations of *Salvia officinalis* extract on the maximum quantum yield of PS II – F_v/F_m in seedlings irradiated at a dose of 200 Gy. Statistically significant difference from control level ($p < 0.05$).

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ШАМІЛОВ Е. Н.[1], АБДУЛЛАЄВ А. С.[1], ШАМІЛЛІ В. Е.[1], АЗІЗОВ І. В.[2]
[1] Інститут Радіаційних Проблем Міністерства Науки та Освіти Азербайджанської Республіки,
Азербайджан, AZ1143, м. Баку, Б. Вахабзаде, 9
[2] Інститут Молекулярної Біології та Біотехнології Міністерства Науки та Освіти Азербайджанської Республіки,
Азербайджан, AZ1073, м. Баку, просп. Матбуат, 2А

ВИВЧЕННЯ СКЛАДУ ТА ПРОТИРАДІАЦІЙНИХ ВЛАСТИВОСТЕЙ ШАВЛІЇ ЛІКАРСЬКОЇ (*SALVIA OFFICINALIS* L.) НА НАСІННІ ПШЕНИЦІ

Мета. Ціль дослідження полягала у вивченні хімічного складу, розробці способів отримання екстракту з листя *Salvia officinalis* L. та вивчення їх протирадіаційних властивостей на опроміненому насінні пшениці сорту Гюнешлі. ***Методи.*** За допомогою якісних реакцій і хроматографії в рослинах *S. officinalis* встановлено вміст ліпідів, ефірних олій, дитерпенових кислот, фенольних сполук, макро- і мікроелементи. Насіння пшениці опромінювали на установці URI (K-25) за потужності 13,4 рад/с у дозі 200 Гр. Кількість хлорофільних пігментів, каротиноїдів, малонового диальдегіду вимірювали на спектрофотометрі. Флуоресценцію хлорофілу в листках визначали за допомогою приладу MINI-PAM. ***Результати.*** За результатами морфологічних і фізіолого-біохімічних показників проростків, 0,01 % і 0,001 % екстракти *S. officinalis* позитивно впливають на ріст і розвиток, підвищують максимальний квантовий вихід ФС II і знижують вихід продукту перекисного окислення ліпідів. ***Висновки.*** Встановлено, що екстракт *S. officinalis* має радіозахисну дію і може використовуватися як радіопротектор для деяких сільськогосподарських рослин.
Ключові слова: гамма-ізоляція, малоновий діальдегід, фотосинтетичні пігменти, ріст і розвиток, *Salvia officinalis* L.