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EVALUATION OF THE PROTECTIVE EFFECT OF TOTAL EXTRACTS OF BURDOCK AND SAGE IN IRRADIATED WHEAT SEEDS

Aim. The aim of the study was to obtain total extracts from sage leaves (*Salvia officinalis* L.) and burdock roots (*Arctium lappa*) and to study their antiradiation properties in sprouts obtained from irradiated «Bereketli-95» wheat seeds. ***Methods***. Alcohol extracts from sage leaves and burdock roots were prepared by multiple extraction. Extraction was performed with 70% ethyl alcohol in a water bath with a reflux condenser. Wheat seeds treated with aqueous solutions of total extracts were irradiated at a dose of 200 Gy using a URI (K-25) setup at a dose rate of 5.213 rad/sec. The amount of *Chl a*, *Chl b*, carotenoids and malonic dialdehyde was determined using a Multiscan GO spectrophotometer. Chlorophyll fluorescence was determined using a photosynthetic yield analyzer (Mini-pam, Germany). ***Results.*** 0.01% concentration of the total extract improves the morphological parameters of sprouts, enhances photosynthetic activity and maximum quantum yield of PS II, and reduces the yield of the lipid peroxidation product, malondialdehyde. When comparing the therapeutic effect of the extract with the preventive one, it becomes clear that the therapeutic effect is small. ***Conclusions.*** The total extract can be used as a radioprotector for some agricultural crops growing in contaminated soils.

Keywords: total extract, gamma irradiation, malondialdehyde, photosynthetic pigments.

It is known that, after the absorption of ionizing radiation, accompanied by physical changes in cells, chemical and biological processes occur, which lead, first of all, to damage to critical biomolecules in the cell. In this case, the final manifestation of biological damage can stretch for hours, days and even decades. Therefore, the search for new sources of biologically active drugs in order to create highly effective radioprotective agents is an urgent problem.

Currently, radiation protection agents prepared from natural raw materials are widely used, in particular, from plants that contain a whole complex of physiologically active substances: flavonoids, carotenoids, tocopherols, fat and water-soluble vitamins, which have adaptogenic, immunomodulatory, antioxidant properties and provide, in combination, an increase in the body's radioresistance.

The protective action of the above plant substances is based on the prevention of tissue damage by water radiolysis products. Thanks to various biologically active substances of plants – phenolic (dihydroquercetin, catechins, anthocyanins, flavonols), unsaturated compounds (carotenoids – beta-carotene, lycopene; fatty acids), as well as saturated fatty acids, polysaccharides, alkaloids, triterpene saponins, chlorophylls and other pigments, the processes of damage are slowed down. This allows an increase in the general non-specific resistance of the organism, stimulation of the endogenous background of radioresistance (a complex of endogenous biologically active compounds: amines, thiols and other antioxidants that perform protective functions and suppress the accumulation of excess radiation peroxidation products that are harmful to living cells). The action of these substances is realized in their ability to restore energy and plastic metabolism by binding free radicals and suppressing hyperoxidative catabolic processes, the ability to restore the structure of DNA, mitochondria, cytoplasmic membranes, lysosomes, and increase the synthesis of macroergic compounds. In addition to the stimulating effect on bioenergetics, these substances are able to regulate the transport of ions Ca^{2+}, K^{+}, Na^{+}, Cl^{-} by restoring the structure of

proteins and dipole water molecules, and regulate the synthesis of nucleic DNA and RNA.

To increase the overall resistance of the body, drugs, so-called adaptogens, are widely used. Adaptogens are able to stimulate an increase in the level of endogenous background radioresistance, activate antioxidant, reparative processes, and mobilize other protective resources of the body. Among them, herbal preparations obtained from plants are of particular interest, since they are more accessible, are usually non-toxic, and therefore their use does not cause side effects. Evidence of this is the successful use of Ginseng, Eleutherococcus, and Chinese magnolia vine preparations to increase both the overall resistance of the body to adverse factors and the radioresistance of the body [1, 2]. The authors of this article also studied the anti-radiation properties of extracts of various medicinal plants – saffron, dandelion, collections of various medicinal plants [3, 4, 5], including *Salvia officinalis* L. [6], common in Azerbaijan.

In this paper, we present the results of the protective properties of total extracts of burdock and sage when exposed to gamma radiation.

It is known that burdock root (*Arctium lappa*) is an official medicinal raw material and is recommended as a diuretic. In folk medicine, extracts from burdock leaves and roots are used as a diuretic, diaphoretic, blood-purifying and anti-inflammatory agent. In the works of a number of authors, the antiulcer, adaptogenic, antitumor effect of ethanol and dichloromethane extracts of burdock roots on experimental malignant tumors in animals has been proven [7]. It has been established that concentrated juice obtained from fresh burdock roots stops genetically programmed cell death, growth and division of tumor cells [8].

Considering the above, it seems interesting to study the anti-radiation properties of total extracts of burdock and sage when exposed to gamma irradiation.

The aim of the research was to obtain total extracts from the leaves of sage (*S. officinalis*) and the roots of burdock (*A. lappa*) and to study their antiradiation properties of sprouts obtained from irradiated seeds of the wheat variety «Bereketli-95».

Material and methods

During the experiments, the used sage leaves and burdock root were purchased from the pharmacy network «Herba Flora» in filter bags. Seeds of wheat variety «Bereketli-95» were received from the Institute of Agriculture of the Ministry of Agriculture of the Republic of Azerbaijan.

Alcohol extracts from sage leaves and burdock roots were prepared by multiple extraction. The ratio of raw material to extractant was 1:20. Extraction was carried out with 70% ethyl alcohol while heating in a water bath with a reflux condenser. The extraction process lasted 3 hours while stirring with a magnetic stirrer.

For the preparation of aqueous extracts, we used the technology of the State Pharmacopoeia: infusion in a boiling water bath for three hours, with constant stirring with a magnetic stirrer and subsequent cooling at room temperature [9]. Water was evaporated from the obtained extracts at 40°C in a vacuum.

At the second stage of the experiments, the antiradiation activity of total extracts of burdock and sage was studied on sprouts of wheat of the Bereketli-95 variety. The seeds were treated in aqueous solutions of total extracts of burdock and sage for 8 hours. After drying, the wheat seeds were irradiated at a dose of 200 Gr using the URI (K-25) installation at a dose rate of 5.213 rad/sec, the time required for irradiation at a dose of 200 Gr was 63 minutes 56 seconds, the irradiation source was ^{60}Co. The laboratory planted wheat seeds in plastic pots in 4 replicates. To determine the growth dynamics, the growth length of the seedlings was measured 2 times a week for 40 days.

At the same time, we planted irradiated seeds in an experimental field under field conditions. To study the degree of radiation damage and the degree of radioprotection of total extracts on sprouts, the amount of photosynthetic pigments – chlorophylls, carotenoids and malonic dialdehyde was measured using a Multiscan GO spectrophotometer (Germany). Pigments were extracted from leaves with 96 % ethyl alcohol and chlorophyll *a*, chlorophyll *b* and carotenoids were determined by absorption spectra. The pigment content was calculated using the Winterman method based on the wet weight of leaves (mg / g). Fluorescence of chlorophylls in leaves was determined on a Photosynthetic Yield Analyzer device (Mini-pam, Germany). Using the formulas $Fv=Fm-F_0$ and Fv/Fm, the efficiency of photochemical energy convercion in photosystem II was calculated.

To determine the content of malondialdehyde (MDA), freshly collected plant leaves were

homogenized with 5 % trichloroacetic acid and then centrifuged (12,000 g) for 10 min at 27°C. Equal volumes of the supernatant and 0.5% thiobarbituric acid were then added to 20% trichloroacetic acid and incubated at 96°C for 30 min and quickly cooled in an ice bath. After centrifugation at 12,000 g for 10 min, the optical density of the supernatant liquid was determined at 532 and 600 nm. Data and statistical analysis were performed using Microsoft Excel. Statistical analysis was performed using the Stat Graphics Plus 5.1 statistical package. Mean values were compared using Duncan's multiple test (p=0.05) [6].

Results and discussion

In field conditions, the effect of different concentrations (0.1%, 0.01% and 0.001%) of solutions of total extracts of burdock and sage, first before irradiation (preventive effect), and then after irradiation (as a therapeutic agent) on the growth and photosynthetic activity of seedlings from irradiated seeds of the Bereketli-95 wheat variety was studied.

To study the prophylactic effect of total extracts, the seeds were treated with extracts in three concentrations (0.1%, 0.01% and 0.001%), and then irradiated at a dose of 200 Gr. To determine the morphological and physiological-biochemical changes in the sprouts, the seeds were planted in five variants and four replicates. The first was intact, the second was irradiated, the third was treated with a 0.1 percent solution of total extracts before irradiation, the fourth was treated with a 0.01 percent solution of total extracts and the fifth was treated with a 0.01 percent solution of total extracts. After germination of the planted seeds, visual observations were made and the height and dynamics of seedling development were recorded every 4 days. After analyzing the results, it was found that the seedlings treated with the last two concentrations were taller and developed better. In the first 10 days of development, the effect was not particularly noticeable, and after 40 days, the seedlings treated with a 0.01% extract solution showed better growth results. Towards the end of the growing season, the length of the seedlings almost equaled the control variant and in some cases even exceeded them. The results are shown in Fig. 1.

There are different methods for determining the degree of plant damage. One of these methods is the thiobarbiturate method. As is known, under the influence of free radicals formed, peroxidation of cellular membrane lipids occurs. One of the end products of peroxidation is malondialdehyde (MDA), which is an indicator of the degree of damage to the entire organism [10].

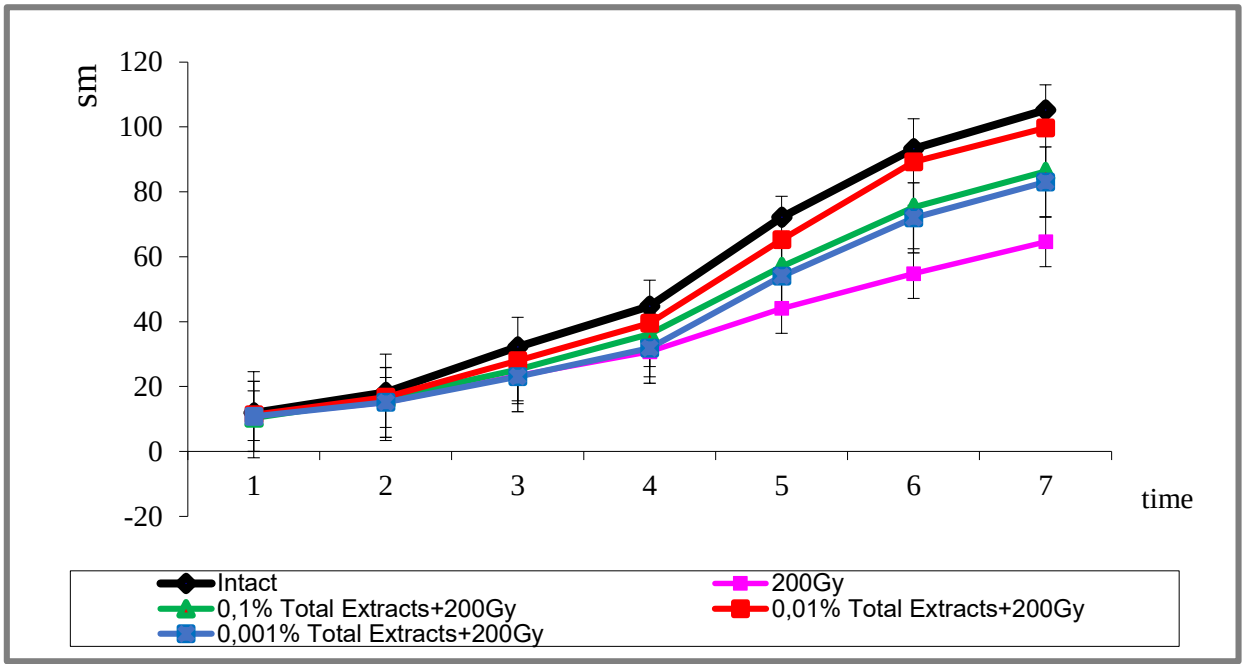

Fig. 1. Growth dynamics of intact wheat sprouts and those treated with 0.1%, 0.01% and 0.001% total extract solution. *Statistical difference in different variants ($p \geq 0.01$).

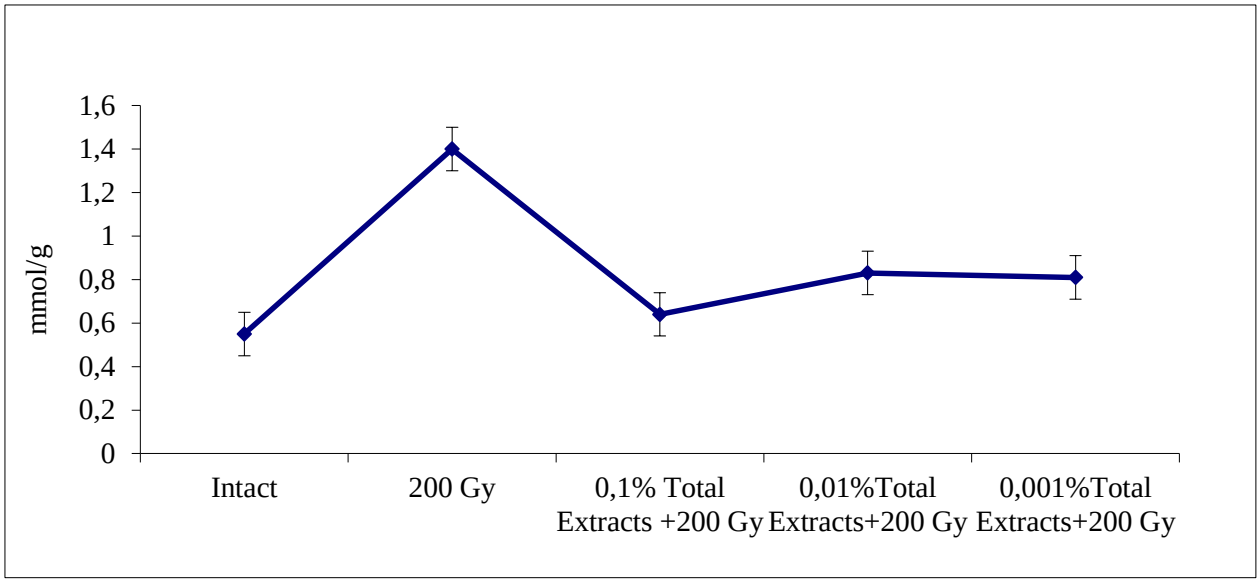

Fig. 2. The amount of malonic dialdehyde in wheat sprouts irradiated and treated with different concentrations of the total extract in different percentages (mmol/g). *Statistical difference in different variants ($p \geq 0.01$).

As shown in Fig. 2. as a result of irradiation in sprouts, the products of lipid peroxidation increase almost threefold, and the solutions of the extract, depending on the concentration, reduce the products of MDA by about 30–35 percent.

Based on the results of the experimental data obtained, we conclude that in comparison with the irradiated version, the total extract improves the morphometric parameters of sprouts and reduces the yield of the product of lipid peroxidation oxidation of malondialdehyde.

As is known, the course of photosynthesis also depends on the content of chlorophyll pigments. With a greater amount of photosynthetic pigments in the cells, the color of the plants becomes bright green. Under the influence of radiation, which is one of the stress factors of plants, the amount of chlorophyll pigments decreases. Therefore, to study the degree of damage to plants by radiation and the radioprotective properties of the total extract, in addition to morphological indicators, it was necessary to determine the amount of pigments and carotenoids in the sprouts of gamma-irradiated wheat. Previously conducted studies have shown that extracts from various medicinal plants and their compositions, as well as various biogenic metal complexes activate antioxidant enzymes, increase the amount of chlorophylls and, at the same time, lead to an increase in the protective and adaptive properties of irradiated plants [11, 12, 13]. The change in the amount of chlorophyll pigments and carotenoids depending on the percentage of total extract in irradiated sprouts is shown below.

As shown in Fig. 3, with irradiation at a dose of 200 Gy, the amount of chlorophylls *a* and *b* decreases (chlorophylls b is two times more) and carotenoids, and in the variant with the total extract, the total extract increases the amount of chlorophyll pigments and carotenoids. If we compare all the tested concentrations, it will be seen that 0.01% total extract increases the amount of pigments even more.

In order to study the effect of the total extract on photosynthetic activity, we determined the maximum quantum yield of photosystem II (PS II) in irradiated and treated with different concentrations of the total extract in wheat sprouts. Fluorescent methods are based on the fact that chlorophylls in photosynthetic processes serve as a natural indicator of the state of green plant cells. Under unfavorable environmental conditions, including exposure to radiation, changes in chlorophyll fluorescence occur. By determining these changes as a source of information, specific conclusions can be made on the course of the photosynthesis process. In order to determine the maximum efficiency of PS II, the index of maximum quantum efficiency of PS II – Fv/Fm is used. And this indicates the potential quantum efficiency of PS II. A decrease in this value means

a stressful state and partial damage to PS II in plants [14].

The radioprotective effect of the total extract is similar to the effect of previously studied extracts from various medicinal plants and their various compositions in wheat sprouts irradiated with different doses.

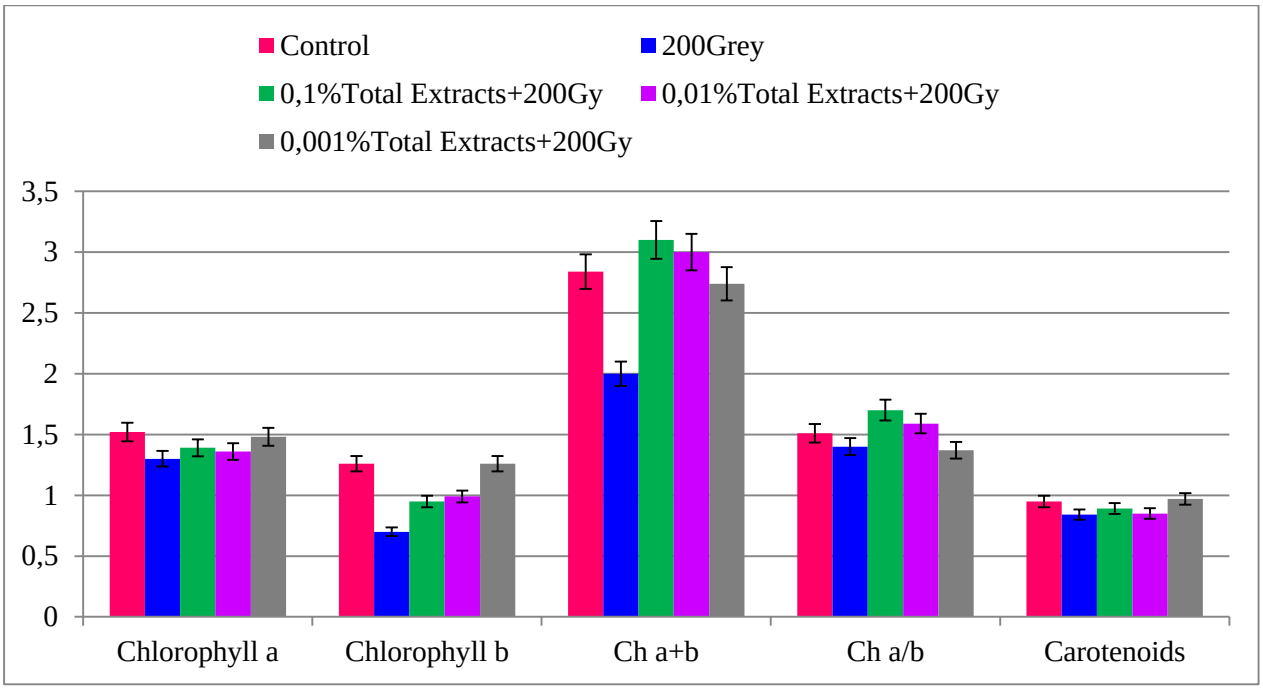

Fig. 3. Effect of total extract on the yield of chlorophyll pigments and carotenoids in irradiated wheat sprouts (mg/g) at a dose of 200 Gy. Experimental variants – control, irradiated, treated with 0.1% total extract, 0.01% total extract and 0.001% total extract. *Statistical difference in different variants ($p \geq 0.01$).

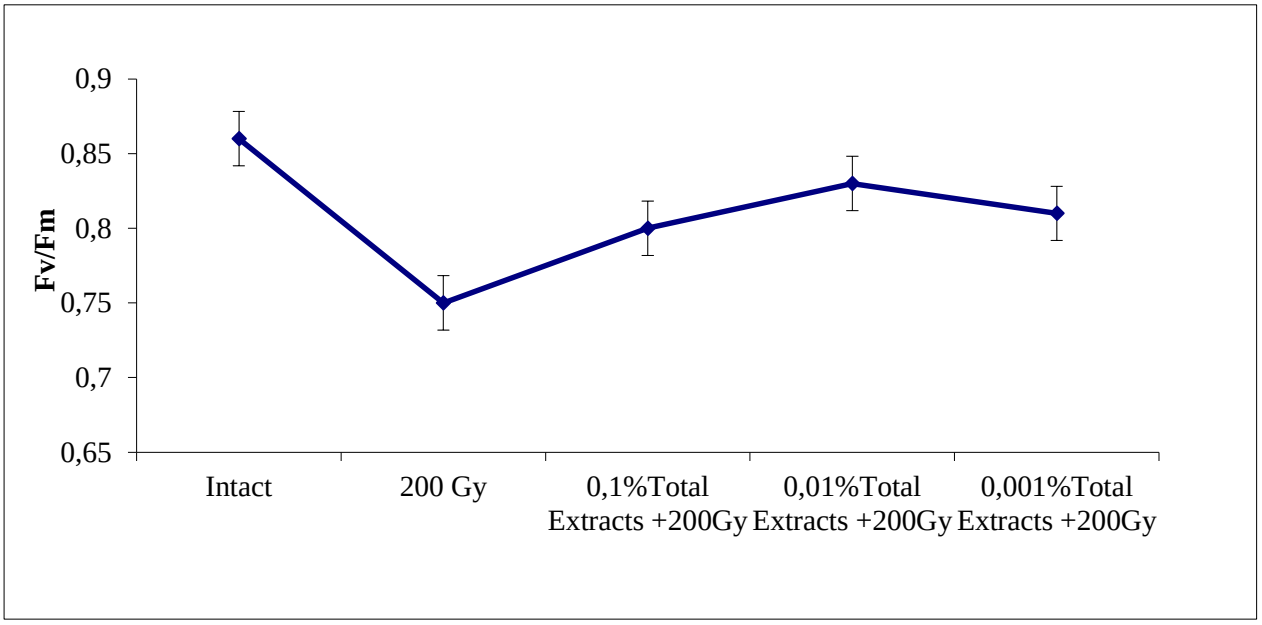

Fig. 4. Change in the maximum quantum yield of PS II under the action of solutions of different concentrations of the total extract in irradiated seedlings. *Statistical difference in different variants ($p \geq 0.01$).

As shown in Fig. 4, irradiation at a dose of 200 Gy maximally reduces the photosynthetic activity of wheat seedlings, and the total extract, especially at a concentration of 0.01%, enhances photosynthetic activity and the maximum quantum yield of PS II within the intact variant.

In order to compare the preventive and therapeutic effect of the total extract, experiments were conducted to study the therapeutic effect of the extract. To study the therapeutic effect of total extracts, Bereketli-95 wheat grains were irradiated at a dose of 200 Gy. Then, the irradiated grains were treated with the same concentrations of total extracts (0.1%, 0.01% and 0.001%), planted in laboratory conditions and monitored for growth and development, determined the amount of photosynthetic pigments and the amount of malonic dialdehyde, as well as the maximum quantum yield of PS II in wheat sprouts. When comparing the therapeutic effect of the extract with the preventive one, it becomes clear that, although the therapeutic effect is small, the restorative trend in all parameters is preserved.

Conclusıons

According to field and laboratory experiments, it has been established that the total extract at a concentration of 0.01%, protecting against the negative effects of radiation, normalizes growth and development, photosynthetic activity, and also reduces the process of lipid peroxidation of cell membranes in sprouts. The extract can be used as a radioprotector for some agricultural crops growing on contaminated soils.

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ОЦІНКА ЗАХИСНОГО ЕФЕКТУ СУМАРНИХ ЕКСТРАКТІВ ЛОПУХА ТА ШАЛФЕЯ ЛІКАРСЬКОГО В ОПРОМІНЕНОМУ НАСІННІ ПШЕНИЦІ

Мета. Метою дослідження було отримання загальних екстрактів з листків шавлії (*Salvia officinalis* L.) та коренів лопуха (*Arctium lappa* L.) та вивчення їх протирадіаційних властивостей у паростках, отриманих з опроміненого насіння пшениці сорту «Берекетлі-95». ***Методи.*** Спиртові екстракти з листків шавлії та коренів лопуха готували методом багаторазової екстракції. Екстракцію проводили 70% етиловим спиртом на водяній бані зі зворотним холодильником. Насіння пшениці, оброблене водними розчинами загальних екстрактів, опромінювали дозою 200 Гр за допомогою установки URI (K-25) з потужністю дози 5,213 рад/с. Кількість Хл a, Хл b, каротиноїдів та малонового діальдегіду визначали за допомогою спектрофотометра Multiscan GO. Флуоресценцію хлорофілу визначали за допомогою аналізатора фотосинтетичної продуктивності (Mini-pam, Німеччина). ***Результати.*** Концентрація 0,01% загального екстракту покращує морфологічні параметри паростків, підвищує фотосинтетичну активність та максимальний квантовий вихід PS II, а також зменшує вихід продукту перекисного окислення ліпідів, малонового діальдегіду. При порівнянні терапевтичного ефекту екстракту з профілактичним, стає зрозуміло, що терапевтичний ефект невеликий. ***Висновки***. Загальний екстракт може бути використаний як радіопротектор для деяких сільськогосподарських культур, що ростуть на забруднених ґрунтах.

Ключові слова: сумарний екстракт, гамма-опромінення, малоновий діальдегід, фотосинтетичні пігменти.