PROCEEDINGS OF THE LATVIAN ACADEMY OF SCIENCES. Section B, Vol. 72 (2018), No. 1 (712), pp. 38–42.
DOI: 10.1515/prolas-2018-0003

INFLUENCE OF A MODIFIED PLANT EXTRACT ON ACTIVITY OF ANTIOXIDANT ENZYMES AND CONCENTRATION OF PIGMENTS IN GAMMA-IRRADIATED PLANTS OF MAIZE AND WHEAT

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Communicated by Isaak Rashal

*The influence of a medicinal plant extract, immobilised by ligands, on the activity of antioxidant enzymes and photosynthetic pigment concentration of wheat and maize was studied. The object of study was seed of drought-resistant firm durum wheat (*Triticum durum *Desf.) and maize (*Zea mays *L.). Seeds were subjected to general uniform γ-radiation from a ^{60}Co source on a Rkhund installation at average dose power of MD = 0.306 Gy/sec. Before radiation seeds were treated in modified extract from medicinal plants. The treatment of seeds with 0.1 and 0.01% solution of modified extract from* Hypericum, Dandelion*, and* Calendula *caused significant reduction in processes initiated by radiation and in formation of free radicals. On the basis of the obtained results it was concluded that the used modified plant extract collection had a protective effect, reducing the amount of free radicals produced by γ-irradiation.*

Key words: *superoxide dismutase, catalase, malondialdehyde, chlorophyll, carotenoid.*

INTRODUCTION

It is common knowledge that γ-radiation has a damaging effect on living organisms. Under the influence of γ-radiation-induced free radicals, reactive oxygen species that cause oxidative modification of macromolecules are generated causing effect to cellular structures. γ-irradiation damage initiated by free radicals promotes oxidative modification of macromolecules caused by reactive oxygen species, and can result in a loss of the integrity of cellular structures. Active oxygen modifications cause chain reactions with accumulation of lipid, peroxyl and other radicals (Rogozhin *et al*., 2000). In recent years, in the radiotherapy treatment agents obtained from natural sources, in particular, from plants have been used, as plants contain the entire complex of the physiologically active materials: flavonoids, carotenoids, tocopherols, fats, and water-soluble vitamins. These compounds have adaptogenic and antioxidant properties that promote the radio resistance of organisms (Gusha *et al*., 2000). In lipids, mainly in polyunsaturated fatty acids, reactive oxygven species (ROS) cause chain reactions with accumulation of lipid, peroxyl, alkoxyl, and other radicals. Organisms are able to protect themselves from the damaging effects of free radicals due to highly active antioxidant system that includes low and high molecular weight substances capable of inhibiting of free radical processes (Zenkov *et al*., 1993). Low doses of gamma irradiation generally increase germination of seeds, while at high doses the germination of seeds decreases and development of seedlings is suppressed. With increasing radiation dose, the damage repair system is initiated depending on different adaptive strategies (Nikolaeva *et al*.,1985).

Biologically active agents of plants have a number of benefits in comparison with substances of an animal and synthetic origin. They have low toxicity, a wide range of pharmacological activity, ability to optimise the physiological processes proceeding in a human body and to increase natural protection of an organism against environmental effects (Maksyutina *et al*., 1985). Also, plant extract has a complex effect on organisms, as they contain biologically active agents of different groups in concentrated amount. Strength of pharmacological effects of plant extract considerably depends on the natural compounds present (Nikolaeva *et al*., 1985). In earlier studies we described the radio protective properties of extracts obtained from several medicinal

plants growing in the territory of Azerbaijan (Shamilov *et al.*, 2014).

In the present work the action of the immobilized ligands in medicinal plant extract on the activity of antioxidant enzymes and photosynthetic pigment concentration of wheat and maize was studied.

MATERIALS AND METHODS

We used seeds of drought-resistant firm *Triticum durum* wheat and maize of *Zea mays* L. as the object of the study. Seeds were subjected to general uniform γ-radiation from a ^{60}Co source on a Rkhund installation at average power of radiation dose MD = 0.306 Gy/ sec. Prior to irradiation seeds were treated in modified extract from medicinal plants. Crushed dry plant material consisting of *Hypericum*, dandelion roots and calendula flowers (mass ration 1 : 1 : 1) was extracted in 70% ethanol at ratio 1 : 15 at room temperature with constant stirring. The solution was then filtered, and the extraction procedure was repeated three times with fresh solvent. The collected filtrated was then combined and evaporated on a rotational evaporator in vacuum. To obtain a high-molecular modified composition, the total extract of collection of plants and PVP in the ratio 1 : 4 was used. The process was carried out in a flask, stirring at temperature 60 °C for two hours. The obtained product was dried in air until mass was stable.

The Electron Paramagnetic Resonance (EPR) method was applieed to determine radicals in gamma irradiated seeds.

The activity of superoxide dismutase was determined by the ability to inhibit autoxidation of adrenaline in an alkaline environment. The rate of reaction was evaluated spectrophotometrically, in solution with and without extract, as the optical density of the accumulated auto-oxidation product of adrenaline, which has absorption at a wavelength of 347 nm (Sirota, 2000).

The activity of catalase was determined on a polarograph (OH103) with Clark electrode by release of oxygen.

The concentration of malondialdehyde was determined spectrophotometrically (Multiscan GO, Germany) at wavelength 532 nm, using thiobarbituric acid.

The concentration of chlorophyll was determined on a spectrophotometer (Multiscan GO, Germany) by measurement of optical density at wavelength 665 nm and 649 nm.

Data analysis and statistical analysis was conducted using Microsoft Excel. Statistical analysis was performed with the aid of the Statgraphics Plus 5.1 statistical package. The means of values were compared by Duncan's multiple range test (p = 0.05).

RESULTS

Figure 1 shows the EPR spectra of radicals in wheat and maize. Lower amounts of radicals were by treating seed with 0.1 and 0.01% solutions of modified extract (Hypericum, Dandelion, Calendula) before γ-irradiation.

Regarding products of oxidation of lipids, seedlings of γ-irradiated seeds contained higher amounts of malondialdehyde, than in unirradiated seeds. In seedlings from treated seed the concentration of MDA was similar to that of the control. A significant effect of the extract was found at extract concentration of 0.01% (Figs. 1, 2).

Stressful abiotic factors, especially radiation, promote antioxidant systems for protection against the destroying action of free radicals (Zenkov, 1993). Superoxide dismutase and catalase activity was higher (Figs. 3, 4) in the irradiated plants, and lower activity was observed with treatment with

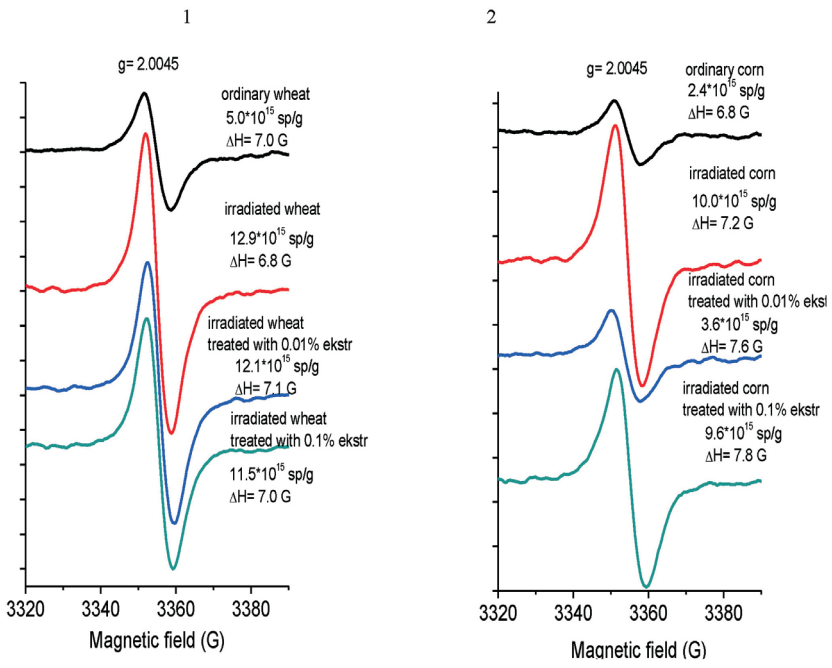

Fig. 1. Electron Paramagnetic Resonance (EPR) spectra of radicals of wheat and maize by (1 and 2). Free radicals were measured using the EPR method. The figure shows that the modified extract at a concentration of 0.01% promoted the maintenance free radical amounts at the control level.

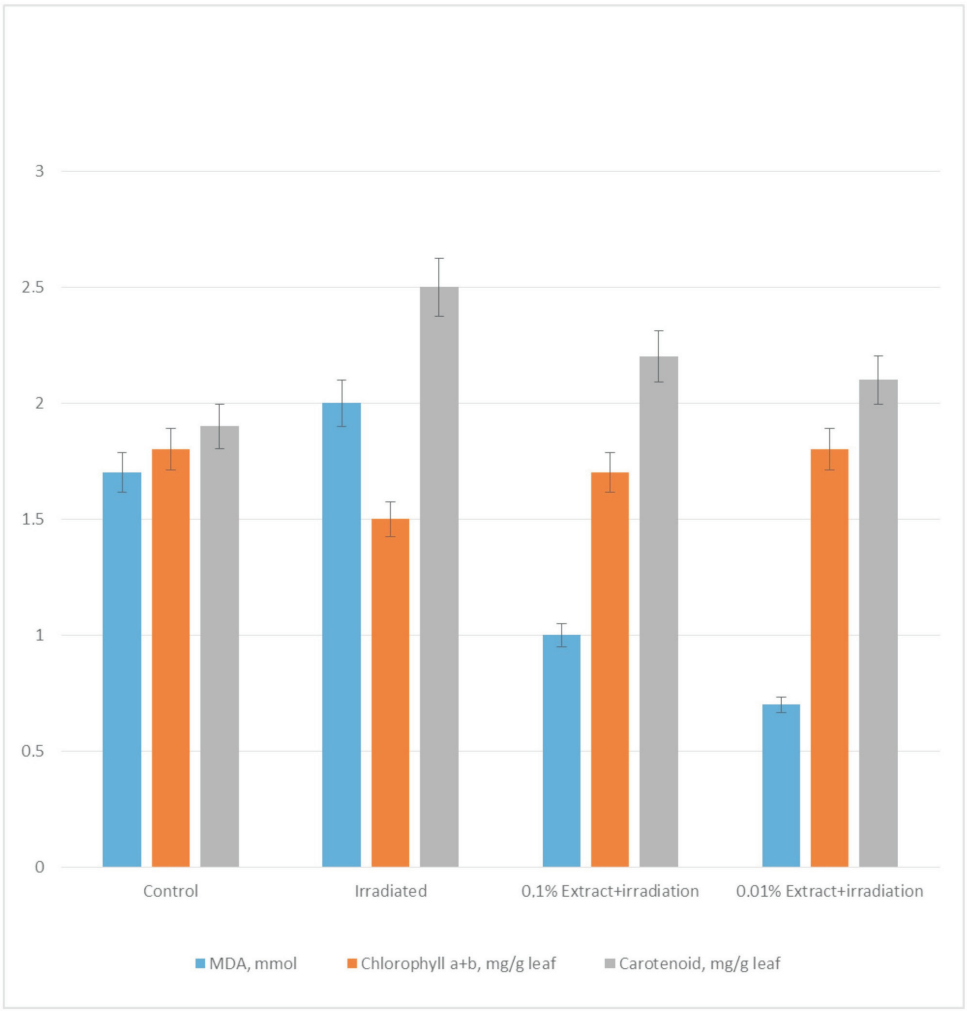

Fig. 2. Influence of plant extract on the level of MDA, chlorophyll and carotenoid in maize plants. The figure shows that a modified extract with a concentration of 0.01% contributed to maintaining the concentration of malondialdehyde, chlorophyll, and carotenoids at the control level. Each bar represents mean ± SD (standard deviation) for average of n = 3 independent experiments, $p = 0.05$.

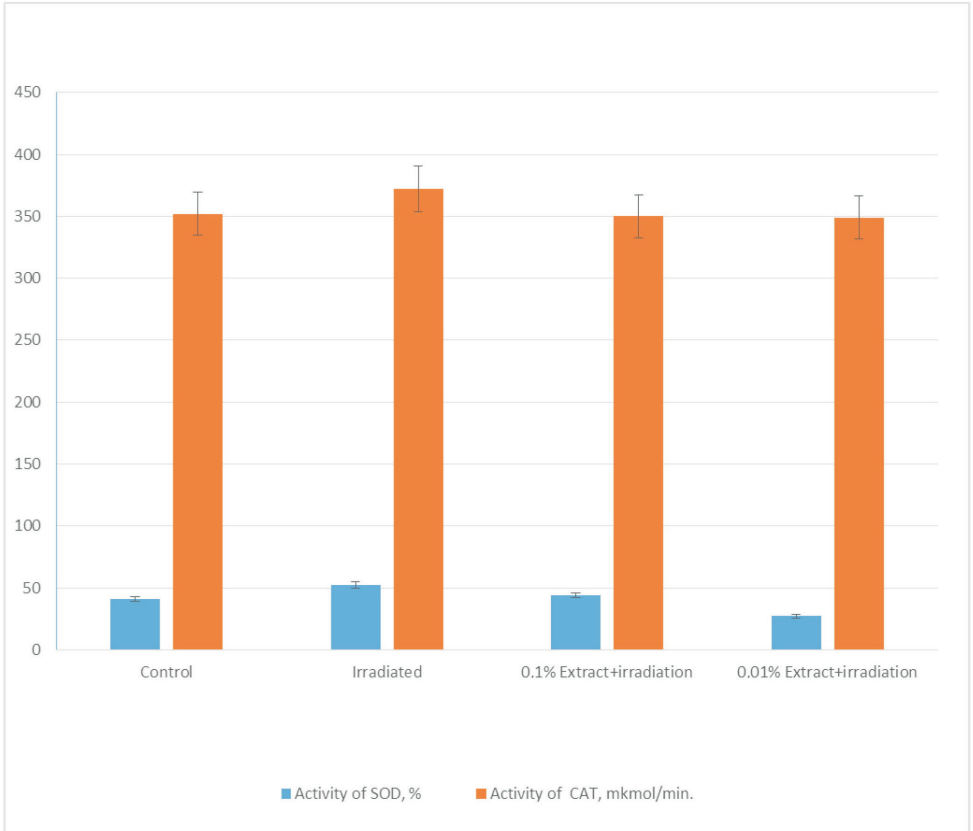

Fig. 3. Influence of extract on the activity of superoxide dismutase (SOD) and catalase (CAT) in wheat plants. The figure shows that a modified extract with a concentration of 0.01% contributes to maintaining the activity of SOD and CAT at the control level in wheat plants. Each bar represents mean ± SD (standard deviation) for average of n = 3 independent experiments, $p = 0.05$.

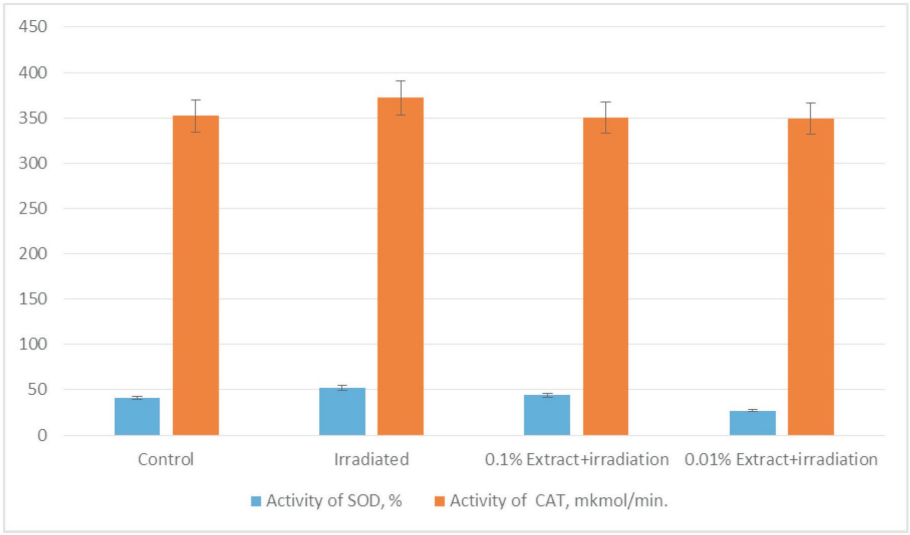

Fig. 4. Influence of extract on the activity of superoxide dismutase (SOD) and catalase (CAT) in maize plants. The figure shows that a modified extract with a concentration of 0.01% contributes to maintaining the activity of SOD and Cat at the control level in maize plants. Each bar represents mean ± SD (standard deviation) for average of n = 3 independent experiments, $p = 0.05$.

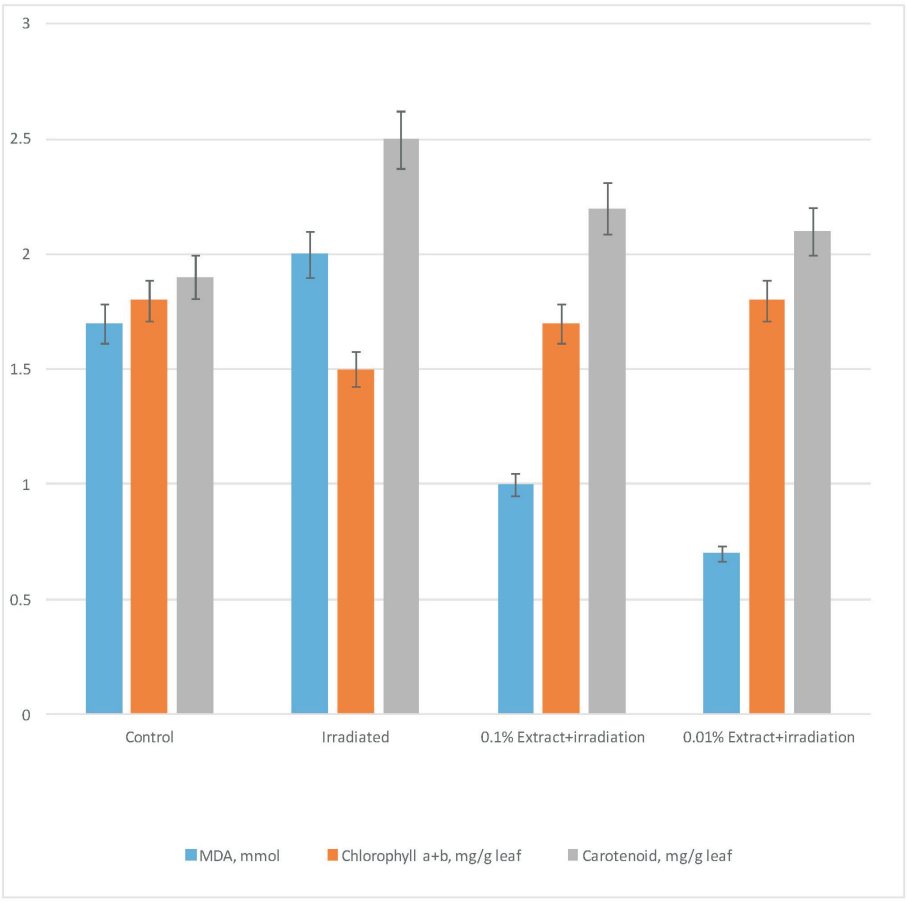

Fig. 5. Influence of extract on the level of MDA, chlorophyll and carotenoid in wheat plants. The figure shows that a modified extract with a concentration of 0.01% contributes to maintaining the concentration of malondialdehyde, chlorophyll, and carotenoids at the control level in wheat plants. Each bar represents mean ± SD (standard deviation) for average of n = 3 independent experiments, $p = 0.05$.

extract, likely due to reduction of quantity of free radicals. The reduction of the concentration of chlorophyll a and b, and slight increase in carotenoid concentration were observed in plants grown from the irradiated seeds, whereas the concentrations of green pigments and carotenoids were at the level of the control variant in plants treated with the extract (Figs. 2, 5).

DISCUSSION

The results obtained in our experiments and previous work suggest that gamma-irradiation may induce lipid peroxidation in leaves. One of the major plant tissue reactions to radiation was expressed in elevated levels of secondary products of peroxidation. It is known that peroxidation of lipids indicates damage of cellular membranes. As a result of peroxidation of lipids final metabolites are formed (lownew dialdehyde, ethane, a pentane, etc.), which react with thiobarbituric acid (Zenkov, *et al.*, 1993). In work carried out with an aqueous extract of *Mentha* different species were studied in their ability to inhibit iron (III)-ascorbate-catalysed hydroxyl radical mediated peroxidation of brain phospholipid *in vitro*. It was observed that the extract of *M. piperita* performed better than the other extracts (Dorman *et*

al., 2003). Studies have also shown that the aqueous extract of *M. Piperita* reduces radiation-induced lipid peroxidation in mouse liver, testis, and blood (Samarth, 2009).

Biological preparations, for example, extracts of ginesing root have adaptogenic radioprotective properties under conditions of chronic exposure (Lee, 2005).

Extracts of *Podophyllum hexandrum* acted in a holistic manner at various levels. Its administration on mice prior to irradiation led to a dramatic increase (~80%) in survival (Raj Kumar *et al*., 2008). At the molecular level, it induces pro-survival protein and DNA repair protein expression while down-regulating the expression of proteins associated with induction of apoptosis. These activities are congruent with the *in vitro* free radical modulating ability of rhizome extracts of *P. hexandrum*.

We showed that the irradiation of seeds in high doses increased the content of hydrogen peroxide, superoxide, and hydroxyl radicals and thus activated lipid peroxidation and resulted in the activation of the antioxidant system of plants.

CONCLUSION

On the basis of the obtained results, it was concluded that modified plant extract at a concentration of 0.01% after γ-irradiation resulted in lower levels of free radicals, and the maintenance of normal concentration of malondialdehyde, chlorophyll, carotenoids, and the activity of superoxide dismutase and catalase had the protective effect, reducing the amount of free radicals.

REFERENCES

Dorman, H. J., Koşar, M., Kahlos, K., Holm, Y., Hiltunen, R. (2003). Antioxidant properties and composition of aqueous extracts from *Mentha* species, hybrids, varieties, and cultivars. *J. Agric. Food Chem*., **51**, 4563–569.

Gusha, N. I., Perkovskaya, G. Yu., Dmitriev, A. P., Grodzinsky, D. M. (2000). Influence of chronic exposure on the adaptive capacity of plants. Radiation Biology [Гуща, Н. И., Перковская, Г. Ю., Дмитриев, А. П., Гродзинский, Д. М. Влияние хронического облучения на адаптивный потенциал растений. Радиационная биология]. *Радиоэкология* [*Radioecology*], **42** (2), 155–158 (in Russian).

Lee, T. K., Johnke, R. M., Allison, R. R., O'Brien, K. F., Dobbs, L. J., Jr. (2005). Radioprotective potential of ginseng. *Mutagenesis*, **20** (4), 237–243.

Maksyutina, N. P., Komissarenko, N. F., Prokopenko, A. P., Pogodina, L. I., Lipkan, G. N. (1985). *Herbal Remedies* [Максютина, Н. П., Комиссаренко, Н. Ф., Прокопенко, А. П., Погодина, Л. И., Липкан, Г. Н. Растительные лекарственные средства]. Zdorov'ja, Kiev. 290 pp. (in Russian).

Nikolaeva, M. G., Razumova, M. V., Gladkova, V. N. (1985). *Directory on Germination of Dormant Seeds* [Николаева, М. Г., Разумова, М. В., Гладкова, В. Н. Справочник о прорастании покоящихся семян]. Nauka, Leningrad. 347 pp. (in Russian).

Raj Kumar, Pankaj Kumar Singh, Rajesh Arora, Raman Chawla, Rakesh Kumar Sharma (2008). Radioprotective activities of Podophyllum hexandrum: Current knowledge of the molecular mechanisms. *Trees Life J*., **4** (1), 1–9.

Rogozhin, V. V., Kuryliuk, T. T., Filippova, N. P. (2000). Changing the reaction of antioxidant system of wheat seedlings after UV irradiation of seeds [Рогожин, В. В., Курилюк, Т. Т., Филиппова, Н. П. Изменение реакции антиоксидантной системы проростков пшеницы после УФ-облучения семян]. *Биофизика* [*Biophysics*], No. 4, 730–736 (in Russian).

Samarth, R. M., Samarth, M. (2009). Protection against radiation-induced testicular damage in Swiss albino mice by *Mentha piperita (*Linn.). *Basic Clin. Pharmacol. Toxicol*., **104**, 329–334.

Sirota, T. V. (2000). Method for determining the activity of superoxide dismutase and antioxidant chemicals. Russian Federation Patent No. 2144674 [Сирота, Т. В. Способ определения антиоксидантной активности супероксиддисмутазы и химических соединений. Патент РФ № 2144674] (in Russian).

Shamilov, E. N., Abdullayev, A. S., Rzayeva, I. A., Muslimova, Z. N., Azizov, I. V. (2014). Comparative study of the radioprotective properties of calendula, chamomile and milfoil. *J. Radiat. Res*., **1** (1), 3–8.

Zenkov, N. K., Menshikova, E. B. (1993). Activated oxygen metabolites in biological systems [Зеньков, Н. К., Меньшикова, Е. Б. Активированные кислородные метаболиты в биологических системах]. *Успехи современной биологии* [*Advances in Modern Biology*], **113** (3), 286–290 (in Russian).

Received 7 November 2016
Accepted in the final form 8 November 2017

MODIFICĒTO AUGU EKSTRAKTU IETEKME UZ ANTIOKSIDANTU ENZĪMU AKTIVITĀTI UN PIGMENTU KONCENTRĀCIJU AR GAMMA-STARIEM APSTAROTOS KUKURŪZAS UN KVIEŠU AUGOS

Pētīta imobilizēto ārstniecisko augu ekstraktu ietekme uz antioksidantu enzīmu aktivitāti un fotosintētisko pigmentu koncentrāciju kukurūzas un kviešu augos, kas izaudzēti no sēklām, kuras tika apstarotas ar gamma-stariem. Parādīts, ka izmantotiem ekstraktiem bija protektīvs efekts, kas samazināja ar apstarojumu inducēto brīvo radikāļu daudzumu.