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ORIGINAL ARTICLE

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Humic Substances Antistress Activity at the Corn Seedlings

Z. H. Muslumova [1]*, I. V. Azizov [2], M. F. Farajov [1]

[1] *Institute of Radiation Problems, Ministry of Science and Education, Republic of Azerbaijan*

[2] *Institute of Molecular Biology and Biotechnologies, Ministry of Science and Education, Republic of Azerbaijan*

*E-Mail: *zohre.biologist@gmail.com*

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Under model conditions, the effect of potassium humate on some indicators in corn seedlings obtained from gamma-irradiated seeds was determined. A physicochemical analysis of potassium humate obtained from pine wood waste was carried out. In field and laboratory experiments, we studied the effect of potassium humate solutions on the morphological and biochemical parameters of corn seedlings obtained from irradiated seeds at doses of 50 and 100 Gy. In all experiments, solutions of potassium humate had a positive effect on the dynamics of growth and development of corn plants grown from irradiated seeds. The effect of potassium humate solutions on chlorophyll and carotenoid pigments, and on the photosynthetic activity (maximum quantum yield of PSII) of plants was studied. It has been established that the treatment of seeds with a 0.1% solution of potassium humate before irradiation reduces the amount of malondialdehyde, a product of lipid peroxidation. Seed treatment with 0.1% and 0.01% solutions of potassium humate before irradiation caused a significant reduction in the harmful effects of ionizing radiation on seedlings of corn plants.

Key words: Zea mays, potassium humate, chlorophyll pigments, irradiated seeds, malonic dialdehyde, radioprotective activity, lipid peroxidation

Humic substances are widely used in agriculture as organic fertilizers. Various salts of humic acids are also used as growth and development stimulants. The stimulating effect of humic compounds on the growth and development of plants, increasing their resistance to adverse environmental factors has been sufficiently studied (Van Stempvoort et al., 2005; Holman, 2002). Soluble forms of humates in small concentrations significantly stimulate the growth and development of plants, increase the supply of nutrients to plants, activate protein and carbohydrate metabolism, and increase crop yields (Stevenson, 1982). We studied the effects of potassium humate on growth and development, on the morphological and physiological parameters of maize (*Zea mays*) variety "Zagatala 68" under conditions of radiation stress. It is known that ionizing radiation ultimately causes a violation of the integrity of cellular structures. The radioprotective properties of potassium humate were studied under model field conditions. There are a lot of scientific works on the use of humic substances in the rehabilitation of contaminated soils (Muslumova, 2015). The use of humic acids with other organic additives has a positive effect on the yield and quality of corn, as well as on soil properties and the availability of elements necessary for plant growth and development (Essam, 2020; Muslumova, 2013). In studies using various concentrations of humate solutions, the highest values of all studied indicators of corn plants were recorded - plant height, shoot diameter (cm), weight of 100 grains (g) and yield index (HI%) (Awwad, 2015). Thus, obtaining and studying the biological activity and radioprotective properties of humic substances is a very relevant direction.

MATERIALS AND METHODS

To obtain humate K, the feedstock - peat - was treated with 3% KOH solution with constant stirring at temperature of 35-40 degrees for 5 hours. After that, the dissolved sodium and potassium humates was separated in a centrifuge. The objects of research were maize seeds of the “Zagatala 68” variety. To study the radioprotective properties of humates, maize seeds were treated with solutions of sodium and potassium humate for 15 hours. Then the seeds were irradiated with doses of 50 and 100 Gy using the URİ (K-25) device. In experiments to study the effect of sodium and potassium humate on the dynamics of growth and development of seedlings were used 0,1%, 0,01% and 0,001% solutions. Plants were grown in the saline environment.

These experiments included a study of the growth dynamics of maize (*Zea mays*) seedlings obtained from irradiated corn seeds at doses of 50 and 100 Gy. Potassium humate was obtained from pine needle compost. We studied the percentage of humus in the compost and the elemental composition of the potassium humate. Before irradiation, maize seeds were treated with humate solutions at the concentration of 0.1% and 0.01%. The amount of chlorophyll a (662 nm), chlorophyll b (644 nm), carotenoids (440 nm) pigments and malondialdehyde (532 nm) determined spectrophotometrically (Multiscan Go, Germany). The maximum fluorescence quantum yield of maize seedlings was measured on a MINI-PAM fluorometer (Germany). Statistical processing of the results was carried out using Microsoft Office Excel and the Statistica software package. Grade the reliability of the obtained results was carried out on the basis of the calculation of the Student's criterion.

RESULTS AND ITS DISCUSSION

Pine needles contain valuable biological components, phytohormones, vitamin C, tannins, anthocyanins, alkaloids, terpenes, macro and microelements - calcium, iron, nickel, cobalt, phosphorus, manganese and zinc. Pine needle essential oil showed remarkable antioxidant activity in scavenging free radicals, in lipid peroxidation, and in reducing power assays. The essential oil showed strong antimicrobial activity with minimal inhibitory concentrations and minimal bactericidal concentrations (Qualls et al., 2003; Xavier de Campos et al., 2014) To obtain potassium humate, we used pine needle compost as a raw material. Pine waste composts are readily available raw materials. They can be harvested from the soil of pine forests. Composts can also be prepared in reactors. This can become a promising technology for the

utilization of organic household and wood waste (Zeng et al., 2012; Zeng et al., 2011). We conducted the physico-chemical analysis of the compost to determine the organic and inorganic composition.

At the beginning of the experiments, we studied the effect of potassium humate solutions on the germination of seeds irradiated at doses of 100 Gy. The germination of seeds not treated with humate solutions decreased to 55-65%. The germination of seeds irradiated at a dose of 100 Gy was 81% on the variant treated with 0.01% humate solutions, 84% on the variant treated with 0.1% potassium humate solutions. We studied the effect of potassium humate solutions on the amount of malonic dialdehyde, an indicator of lipid peroxidation, on chlorophyll pigments and carotenoids, and on the fluorescent indices of leaves (photosystem II) in seedlings.

The results obtained from the experiments performed indicate that the use of potassium humate solutions prevents damage to corn seedlings grown from irradiated seeds, compared with seedlings obtained from irradiated samples not treated with potassium humate solutions. Irradiation of corn seeds at dose of 100 Gy markedly inhibited the development of seedlings. A positive effect was observed in experiments to study the effect of humate solutions on the dynamics of growth and development of plants. Seedlings obtained from seeds treated with a 0.01% solution of potassium humate (PH) showed the best results in all stages of development of growth and development throughout the entire development stage.

As can be seen from Figure 1, growth and development of seedlings obtained from seeds treated before irradiation at a dose of 50 Gy with a 0,1% and 0.01% solutions of the PH increased by 13-15%, especially in the final stages compared to the irradiated variant.

Irradiation of corn seeds at a dose of 100 Gy noticeably inhibits the growth and development of plants (Figure 2) When seeds were treated with 0.1% and 0.01% solutions of the potassium humate (PH) before irradiation at a dose of 100 Gy, a noticeable effect of the complex on the dynamics of plant development was observed. In these experiments, we observed that the growth and development of plants obtained from the seed complex treated with 0,1% and 0.01% solution increased by 15-18% at all stages compared to the irradiated variant.

It is known that radiation stress also affects the biosynthesis of photosynthetic pigments in plants. Many studies showed that chlorophyll pigments in irradiated plants were lower than in control plants. Photosynthetic pigments can be destroyed by gamma rays with a concomitant loss of photosynthetic capacity. In some experiments it was found that gamma irradiation leads to a greater destruction of chlorophyll b than chlorophyll a, due to a violation of its biosynthesis (Ling et al., 2008; Kiong 2008; Strid et al., 1990)

We investigated the effect of the complex on the content of photosynthetic pigments in maize seedlings. The study of the effect of the complexes on photosynthetic pigments was carried out in the spring stage of the experiment in seedlings obtained from seeds irradiated at doses of 100 Gy.

As can be seen from Figure 3, when corn seeds are irradiated with gamma irradiation at a dose of 50 Gy, the amount of photosynthetic pigments chlorophylls a and b decreases by 8-10%. In the irradiated variant of seeds at a dose of 50 Gy, both concentrations of the complex solution equally positively affect the amount of chl a and b and cause an increase in their biosynthesis by 4–6% compared to the irradiated variant. Irradiation at a dose of 100 Gy reduces the amount of chlorophyll a and b by 40% and 50%. In the variant, when the seeds were treated with 0.1% and 0.01% solutions of potassium humate, the amount of chlorophyll an increases by 30–35%, chlorophyll b by 8–12% compared to the irradiated variant. In the variant with seed treatment with 0.01% potassium humate solution, the amount of carotenoids was also higher compared to the irradiated control in both seed irradiation doses. It can be said that the treatment of seeds of corn variety "Zagatala 68" with solutions of potassium humate before irradiation leads to the normalization of the biosynthesis of photosynthetic pigments and carotenoids.

We also studied the effect of potassium humate solutions on lipid peroxidation. Experiments to determine the effect of potassium humate solutions on lipid

peroxidation were carried out at the beginning of the development of corn seedlings in the first weeks. Gamma irradiation induce oxidative stress with overproduction of reactive oxygen species such as superoxide radicals, hydroxyl radicals, and hydrogen peroxides, which react rapidly with almost all structural and functional organic molecules including proteins, lipids, and nucleic acids causing disturbance of cellular metabolism. Reactive oxygen species react with almost all components of the cell. As a result, such an interaction contributes to free radical chain reactions. This ultimately leads to membrane lipid peroxidation (Montiller et al., 2004; Al-Rumaih et al., 2008). The free radicals that appeared after irradiation interact with the lipids of cell membranes. It is known that the process of lipid peroxidation is one of the important indicators of the effect of radiation on living organisms. When free radicals, subsequently formed under the action of radiation, interact with lipids of cell membranes, lipid peroxidation occurs. Lipid peroxidation causes the formation of several end products, one of which is malondialdehyde. The amount of formation of this product determined the degree of damage to the cells. Experiments to determine the effect of potassium humate solutions on lipid peroxidation were carried out at the beginning of the development of corn seedlings in the first weeks. Gamma irradiation induce oxidative stress with overproduction of reactive oxygen species such as superoxide radicals, hydroxyl radicals, and hydrogen peroxides, which react rapidly with almost all structural and functional organic molecules including proteins, lipids, and nucleic acids causing disturbance of cellular metabolism. Reactive oxygen species react with almost all components of the cell. As a result, such an interaction contributes to free radical chain reactions. This ultimately leads to membrane lipid peroxidation (Moghaddam et al., 2020; Murchie et al., 2013)

Figure 4 shows that irradiation at a dose of 100 Gy has a positive effect on the yield of malonic dialdehyde, a product of lipid peroxidation. Seed treatment before irradiation with a 0.1% solution of potassium humate reduces the yield of malondialdehyde by 25-30% in the first weeks. Based on the results obtained, it can be concluded that potassium humate solutions lead to a decrease in the level of the product of lipid peroxidation - malondialdehyde and the maintenance of a normal concentration of photosynthetic pigments.

In the next experiment, we measured the chlorophyll fluorescence in corn leaves to study the effect of potassium humate solutions on the efficiency of photosynthesis (the maximum quantum yield of photosynthesis Fv / Fm).The efficiency of photosynthesis in photosystem II was determined using the formulas $Fv = F_m – F_0$ and F_v / F_m. The chlorophyll fluorescence parameter F_v / F_m reflects the maximum quantum efficiency of photosystem II (PSII). Violations that occur under the influence of stress factors in the primary processes of photosynthesis are reflected in the change in chlorophyll fluorescence in plants. The index of maximum PSII quantum yield – F_v / F_m is used to estimate the maximum efficiency of PSII. This indicator provides information on the potential quantum efficiency of PSII. A decrease in this value may indicate the stress state of the plant and partial damage to PSII. (Kalaji et al., 2014, Delia Marcu et al., 2013).

Table 1: Some chemical characteristics of pine needle compost

pH	Total nitrogen %	Humus %	Nitrogen N/NH4 mg/kg	Phosphorus mg/kg	Potassium mg/kg	K - according to soil gradation mg/kg	Fe- according to soil gradation mg/kg	N according to soil gradation mg/kg	Absorbed Ca mq/ekv	Absorbed Mg mq/ekv
7,0	0,422	9,6	70,55	277,5	376	450- 750	30-60	40-120	28,6	13,9

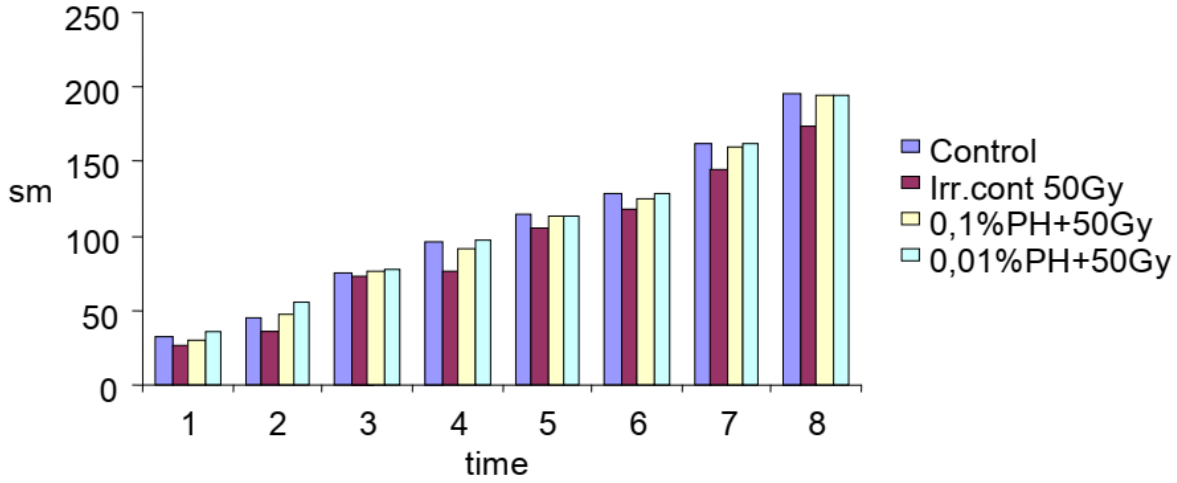

Figure 1. Influence of potassium humate (PH) on the growth and development of corn seedlings of the "Zagatala 68" variety obtained from seeds irradiated at a dose of 50 Gy

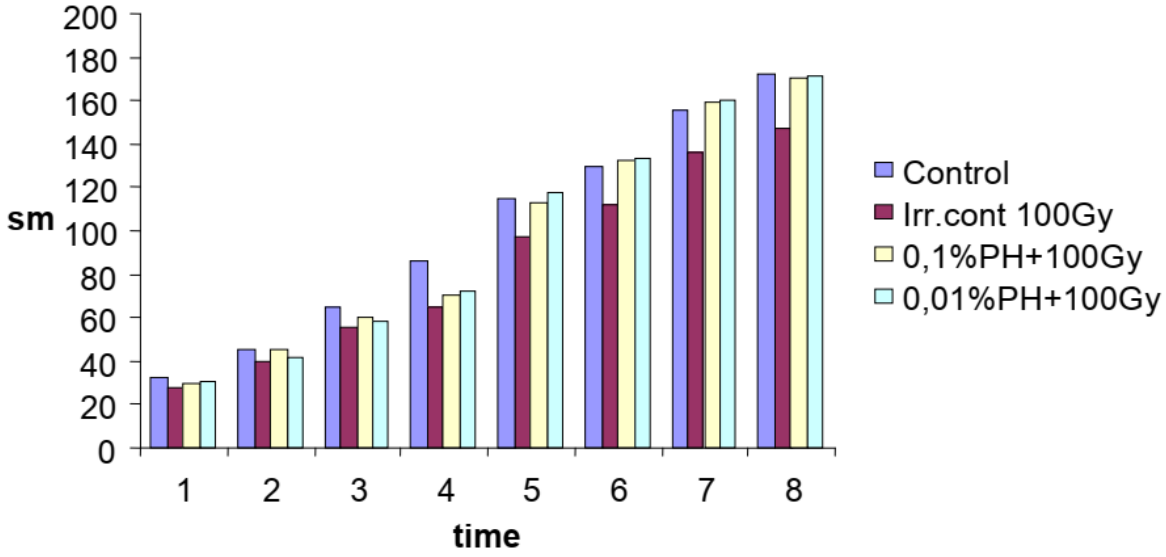

Figure 2. Influence of potassium humate (PH) on the growth and development of corn seedlings of the "Zagatala 68" variety obtained from seeds irradiated at a dose of 100 Gy

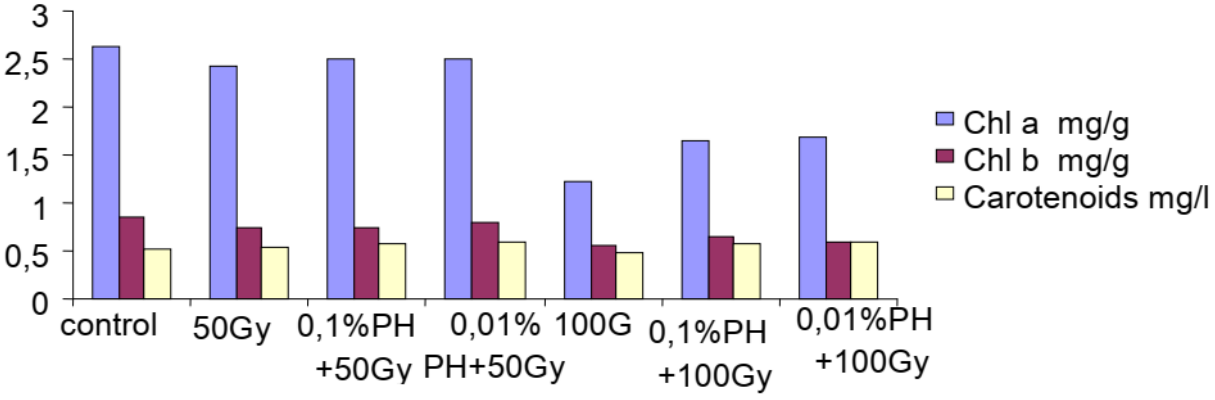

Figure 3. The effect of potassium humate solutions on the amount of chlorophyll pigments and carotenoids in corn seedlings obtained from gamma-irradiated seeds of the "Zagatala 68" variety at a doses of 50 Gy and 100 Gy.

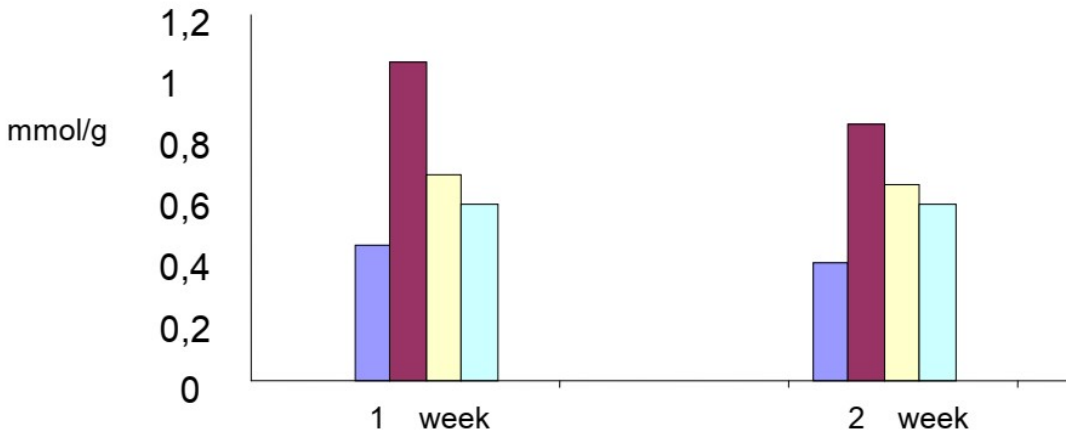

Figure 4. The effect of potassium humate solutions on the amount of malondialdehyde (mmol/g) in 100 Gy irradiated. 1- Cont, 2- Cont. +100 Gy, 3- Cont.+0,1% potassium humat, 4- Cont.+0,01% potassium humat

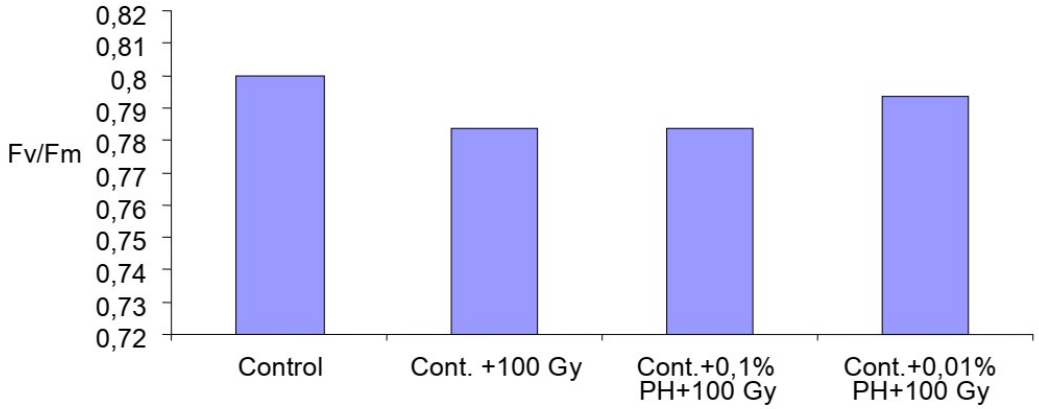

Figure 5. The effect of the potassium humate on the chlorophyll fluorescence parameter F_v / F_m in seedlings obtained from seeds irradiated at the dose 100Gy.

Based on the results of these studies, it can be said that when seeds are irradiated at a dose of 50 Gy, a significant effect of potassium humate solutions on the chlorophyll fluorescence index (F_v/F_m) in plants is not observed. When seeds are treated before irradiation with a 0.01% solution of potassium humate at a dose of 100 Gy, the chlorophyll fluorescence index in plants noticeably increases.

Thus, it can be said that the use of low doses of radiation and optimal concentrations of humate solutions and the creation of technologies on this basis makes it possible to grow agricultural plants under stressful conditions, and also increase plant productivity. In plants treated with 0.01% potassium humate solution, corncobs appeared earlier than others and were larger.

CONCLUSION

Based on these results, it can be concluded that potassium humate solutions lead to decrease in the level of the product of lipid peroxidation - malondialdehyde and the maintenance of a normal concentration of photosynthetic pigments. The positive effect of potassium humate solutions was observed in the study of the development of seedlings obtained from irradiated corn seeds. 0.01% solution of potassium humate showed the highest antistress activity, reducing the harmful effects of gamma irradiation and salt stress.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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