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POSSIBILITIES OF INCREASING THE ANTIOXIDANT PROPERTIES OF GARLIC PLANTS (*Allium Sativum*, L.)

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Abstract: The effect of some selenium-containing compounds on the antioxidant properties of *Allium sativum* L. plants is shown in the present work. Pre-treatment of bulbs before planting and foliar treatment during plant growth with gibberellin solution (125 mg · L^{-1}); potassium selenate (36 µg Se·L^{-1}) and a new cobalt(III) coordinative compound (33 µg Se·L^{-1}) resulted in the increase of the concentration of proline and assimilating pigments, reduced peroxide oxidation of lipids, enhanced antioxidant cell protection. The greatest effect was observed in plants pre-treated with the new coordinative compound, "Fludisec", manifested by an increase of antioxidant properties of leaves and bulbs, optimization of growth process and productivity. X-ray analysis of monocrystal demonstrated that Fludisec is a coordination compound of ionic type tetrafluoroborate-[bis(dimethylglyoximato)-(selenocarbamide)$_{1.4}$-(selenium-seleno-carbamide)$_{0.5}$-(selenium-selenium)$_{0.1}$cobalt(III)] with chemical formula $[Co(DmgH)_2(Seu)_{1,4}(Se\text{-}Seu)_{0.5}(Se\text{-}Se)_{0.1}][BF_4]$.

Keywords: selenium-containing coordination compound, garlic, antioxidant properties, growth process, productivity.

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Introduction

One of the challenges faced by modern agriculture is obtaining plants with improved properties, especially with increased content of microelements, vitamins and biologically active substances which protect against Reactive Oxygen Species (ROS). This need is attributed to rapidly changing climate, resulting in stress conditions for both plants and people, associated with intensive oxidative damage to cells and tissues and emergence of different diseases. Of increasing importance is obtaining products rich in selenium, due to the value of this element in the food chain. Selenium was known since it was discovered by Berzelius in 1817 and initially considered a toxic element. Further studies demonstrated that it is an essential element for human organism, having an important physiological role in the prevention and even the treatment of some diseases. Recently, anti-carcinogenic properties of Se compounds have been widely studied.[1, 2] Selenium is involved in the control and synthesis of prostaglandins, prostacyclins, leukotrienes and tromboxanes. Human genome contains over 20 genes coding for selenoproteins, which is why selenium is an essential component in a diet. Its deficiency in the diet results in the development of different diseases.[3] From the biochemical point of view, selenium is an important biological antioxidant, whose function is explained by the fact that it is a component of glutathione-SH-peroxidase, which, together with other enzymes like superoxide dismutase, catalase, ascorbate peroxidase and vitamin E, protects cellular components from negative effects caused by peroxide accumulation. Together with vitamin E and thio-amino acids, selenium is a liver-protecting factor, now called 3-hepatoprotector factor. Similarly, detoxifying function of selenium is well known: in small concentrations it reduces damaging effect of some toxic ions metals[4, 5]:

Hg^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+}, As^{3+}. The plant-derived organic form of selenium is considered more active and useful for human organism.

Selenium content in food, fruits and vegetables is in direct correlation with its concentration in soil.[6] Worldwide, the soils are generally poor in selenium, with the exception of western regions of Canada, USA, Columbia, Venezuela, Australia, Israel and Ireland. It is noted, that fertilizers containing selenium do not have an evident effect because of soil nitrates, chlorides and phosphates which bind selenium as insoluble compounds. A possible way of increasing biologically available amount of essential elements in edible parts of crops is using fertilizers or physiologically active substances (agronomic bio-fortification) or by selection of crops with increased selenium concentrations (genetic bio-fortification). In this aspect, a number of plant species accumulating this oligoelement was described. In the present decade, the interest in the plants of the genus *Allium*, with the properties of accumulating selenium methylselenocysteine and gamma-glutamyl selenocysteine, which posses well pronounced anti-carcinogenic properties[7, 8], has risen. Garlic (*Allium sativum* L.) has the ability to accumulate microelements, including selenium, but its concentration in garlic cloves depends on its content in soil and on its availability for plants. Recently, some studies regarding supplying such essential oligoelement both with plants and plant-derived pharmaceutical products have been undertaken. In this context, a research work has been carried out to investigate the effect of extra-radical pretreatment of plants with solutions of gibberellin, potassium selenate and Fludisec on biological performance of garlic plants[9]. The objectives of the research were to set up and validate some methods for enrichment of *Allium sativum* L. plants with selenium and to evaluate the potential of antioxidant protection properties of garlic plants after treatment with the compounds.

Results and discussion

$[Co(DmgH)_2(Seu)_{1,4}(Se\text{-}Seu)_{0.5}(Se\text{-}Se)_{0.1}][BF_4]$ was synthesized by the reaction of $Co(BF_4)_2\cdot 6H_2O$ with $DmgH_2$ (dymethylglyoxime) and selenourea in molar ratio of 1:2:2 in aqueous-methanolic media. In this complex, the central atom coordinates two monoanions of dimethylglyoxime ($DmgH^-$), Seu is the molecule of selenocarbamide, Se-Seu is a neutral molecule of selenocarbamide which joined a selenium atom, as well as Se-Se[10].

In the IR spectrum, the absorption bands characteristic of the fragment $Co(DmgH)_2$, cm^{-1} are observed: $\nu_{as}(CH_3)$=2928, $\nu_s(CH_3)$=2871, $\nu_{as}(C{=}N)$=1546, $\delta_{as}(CH_3)$=1461, $\delta_s(CH_3)$=1376, $\nu_{as}(N{=}O)$=1237, $\nu_s(C{=}N)$=1285, $\nu_s(N{=}O)$=1083, $\gamma(OH)$=972, $\delta(CNO)$=730, $\nu_{as}(Co\text{-}N)$=507 and $\nu_s(Co\text{-}N)$=428. The bands $\nu_{as}(BF_4)$=1084, $\nu_s(BF_4)$=761, $\delta(F–B–F)$=524 cm^{-1} can be attributed to ions from external coordination sphere[11]. Interpretation of crystal structure by X-ray methods showed that the compound is of ionic type and consists of a complex cation $[Co(DmgH)_2(Seu)_{1,4}(Se\text{-}Seu)_{0.5}(Se\text{-}Se)_{0.1}]^+$ and anion $[BF_4]^-$. Thus, in the crystal complex, cations $[Co(DmgH)_2(Seu)_2]^+$, $[Co(DmgH)_2(Seu)(Se\text{-}Seu)]^+$ and $[Co(DmgH)_2(Seu)(Se_2)]^+$ with different completion coefficient exist.

Coordination polyhedron of Co(III) is an octahedral consisting of four nitrogen atoms from two monoanions of oximic ligand coordinated in chelating mode and two atoms of selenium placed at axial coordinates, which belong to different ligands: Seu, Se–Seu or Se–Se (Fig. 1).

Appearance of the species Se–Seu and Se–Se can be explained by the fact that mechanisms of formation of elementary selenium are possible for selenocarbamide.[12] Based on some reported studies[13, 14] it can be considered that in case of the ligand Se-Seu, the formation of elementary selenium, followed by its addition to a molecule-radical of selenocarbamide, takes place in the system. In crystal, a complicated system of hydrogen bonds is formed, favored by the presence in the complex cation of amine groups, which act as donors of protons, whereas fluorine atoms and $[BF_4]^-$ anions act as acceptors.

Hormonal system of regulation of biological processes in plant organisms is known to be a unique mechanism, which determines viability and ability to resist the unfavorable environmental conditions. The content of Se in the organism is also a subject of hormonal regulation, however, mechanisms of such regulation are well detailed in mammals[15, 16], and only partially described in plants. The authors demonstrated the effect of epibrasinolideles, heteroauxin, and gibberellin on accumulation of microelements in plant organs. The obtained data showed the ability of both gibberellin and selenium-containing compounds to stimulate the processes of garlic plant growth already at the initial stages of development (Table 1).

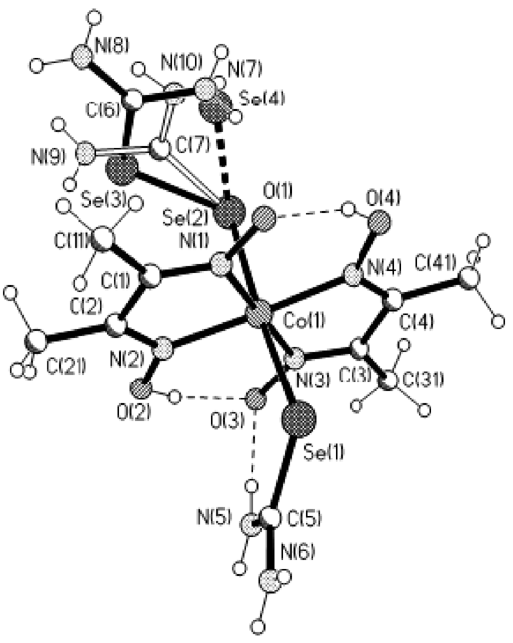

Figure 1. Structure of the complex cation $[Co(DmgH)_2(Seu)_{1.4}(Se\text{-}Seu)_{0.5}(Se\text{-}Se)_{0.1}]^+$.

Table 1. Effect of Se-containing compounds on biomass accumulation by garlic plants.

Variants	Biomass, g					
	Root system		leaves		plant	
	M ± m	Δ, %	M ± m	Δ, %	M ± m	Δ, %
Control	0.25±0.005		0.63 ±0.017		0.88±0.017	
Gibberellin, 125 mg· L^{-1}	0.28±0.006	12.0	0.66 ±0.013	4.76	0.94±0.0015	6.82
K selenate, 0.00001% (35,7µg Se)	0.27±0.005	8.0	0.69 ±0.017	9.52	0.96±0.019	9.09
Fludisec, 0.001%	0.23±0.003	-8.0	0.57 ±0.011	-9.52	0.80±0.020	-9.09
Fludisec, 0.0001%	0.24±0.004	-4.0	0.62 ±0.014	-1.59	0.86±0.024	-2.27
Fludisec, 0.00001% (32.97 µg Se)	**0.29±0.009**	**5.8**	**0.72 ±0.019**	**14.28**	**1.01±0.028**	**14.72**
Fludisec, 0.000001%	**0.26±0.007**	**4.0**	**0.66±0.022**	**4.76**	**0.98±0.031**	**11.36**
Fludisec, 0.0000001%	0.24±0.004	-4.0	0.66±0.018	4.76	0.90±0.019	2.27

In the variant with the use of gibberllin, the weight of the plantlets on the 7^{th} day after germination exceeded the control by 6.82%; pre-treatment with potassium selenate resulted in a 9.09 % increase in plant biomass. The greatest effect was observed in plants treated with coordinative compound Fludisec at 0.00001 and 0.000001%, with 14.72 and 11.36% increase, respectively, compared to the control. The Fludisec treatments resulted in

7.07% increase in the plant weight compared to gibberellin treatment, and in 5.02% increase in the plant weight compared to the treatment with potassium selenate. It supports the idea that selenium causes the increase in biomass due to its participation in chloroplast formation and intensification of photosynthesis[17]. There are data demonstrating that, similar to phytohormons, selenium stimulates the synthesis of green pigments[18].

Plants treated with selenium complexes are characterized by a higher content of assimilating pigments compared to the control (Table 2). Chlorophyll pigments content in leaves of gibberellin-treated plants is 12.5% higher than that of control plants, whereas assimilating pigments content in leaves of the plants treated with Fludisec is 25.0 percent higher.

Table 2. Effect of Se compounds on pigments content in the leaves of garlic plants.

Variants	**Chlorophyll *a*, mg/100 g. fr.w.**		**Chlorophyll *b*, mg/100 g. fr.w.**		**Chlorophyll *a+b*, mg/100 g. fr.w.**		**Carotenoids, mg/100 g. fr.w.**	
	M ± m	**Δ, %**	**M ± m**	**Δ, %**	**M ± m**	**Δ, %**	**M ± m**	**Δ, %**
Control	53.14±0.62		23.19±0.27		76.33±1.41		17.85±0.29	
Gibberellin	59.47±0.84	11.91	26.38±0.24	13.76	85.85±1.18	12.47	20.30±0.24	13.73
K selenate	66.52±0.59	25.17	28.36±0.19	22.29	94.88±0.86	24.30	21.04±0.15	17.87
Fludisec	66.81±0.98	25.72	28.58±0.48	23.24	95.39±1.23	24.97	22.57±0.19	26.44

It should be mentioned that there is a tendency of increase in assimilating pigments content in plants treated with Fludisec, even compared to the plants treated with potassium selenate, and it is especially pronounced when comparing to gibberellin-treated plants (Table 2).

An effect of increase, caused by the application of the solutions of Se-containing compounds, was observed when the level of carotenoids, also having the function of protecting green pigments from oxidative damage, was measured. This phenomenon can be explained by the fact that Se is an antioxidant which activates the defense mechanisms and reduces oxidative stress in plant chloroplasts in stress conditions[19], and participates in the synthesis of chlorophylls and carotenoids[18].

As it is known, one of the common responses to different stress factors, either abiotic or biotic, is a fast generation of reactive oxygen species (ROS), including superoxide radical ($O_2^{\bullet-}$), perhydroxide radical ($HO_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl ($OH^{\bullet}$), peroxyl ($ROO^{\bullet}$) radicals, oxygen singlet ($^1O_2$), organic hydroperoxides (ROOH) etc. It is considered that the increase of ROS is one of the first unspecific reactions of a plant organism to any unfavorable condition, without exceptions.[20,21] Scandalios J.G. (1993) demonstrated that one of the first consequences of the stress produced by extreme factors is appearance of ROS in the cells, oxidative stress and damage to cellular structures.[22] ROS accumulation causes oxidative stress, which, eventually, enhances cell destruction. It was suggested that during different stress types, formation of tolerance is directly related to elimination of ROS.[23, 24] Oxidative stress alleviation is secured by the system of antioxidant protection, an important component of which are antioxidant enzymes which participate in inactivation of superoxide radicals, hydrogen peroxide and hydroxide radicals, as well as by antioxidant compounds with small molecular weight. Due to the antioxidant protection system, in cells in normal conditions there is a dynamic equilibrium of the processes of formation and destruction of ROS.

Some recent works[4, 19, 25] emphasize that Se is an antioxidant that activates the mechanisms of protection and reduces oxidative stress. As a part of a number of functionally active selenoproteins it has function of protection of cells against damage caused by free radicals, hydro-peroxides or lipo-peroxides.[5] Selenium contained in protein molecules is present in a specific set of amino acids. These include such amino acids as selenomethionine, selenocysteine and methylselenocysteine. Selenium is also present in cells as a part of free amino acids (Se-methyl cysteine, seleno-cystathionine, Se-methyl selenomethionine and selenohomocysteine). As known, most of selenoproteins have enzymatic redox functions, which confer to them antioxidant and catalytic activity. In accordance with our data (Table 3) supplementing garlic plants (*Allium sativum* L.) with Se reduces the effect of oxidative stress by decreasing the content of malondialdehyde, reduction of peroxide oxidation of lipids from cell membrane and intensification of the activity of the antioxidant protection enzymes.

Table 3. Content of malondialdehyde and activity of the enzymes of antioxidant protection in garlic (*Allium sativum* L.) leaves and bulbs.

Parameters	Control	Gibberellin		K selenate		Fludisec	
	M ± m	M ± m	Δ, %	M ± m	Δ, %	M ± m	Δ, %
In leaves (after second treatment)							
MDA, $\mu M \cdot g^{-1}$ fr.w.	24.5 ± 0.3	22.9 ± 0.04	-6.8	21.5 ± 0.2	-12.5	21.2 ± 0.4	-13.6
SOD, conv. un.$\cdot g^{-1}$ fr.w.	69.8 ± 0.8	75.7 ± 0.8	9.3	77.6 ± 1.1	12.0	84.3 ± 1.3	21.7
CAT, mM $\cdot g^{-1}$ fr.w.	0.37 ± 0.002	0.6 ± 0.01	62.2	0.6 ± 0.01	62.2	0.7 ± 0.02	89.2
APX, mM $\cdot g^{-1}$ fr.w.	1.9 ± 0.06	2.2 ± 0.03	15.8	2.5 ± 0.05	31.6	2.8 ± 0.02	47.4
GR, mM $\cdot g^{-1}$ fr.w.	27.1 ± 0.5	34.9 ± 0.4	28.6	33.3 ± 0.7	22.9	46.2 ±0.82	70.5
GPX, mM $\cdot g^{-1}$ fr.w.	12.0 ± 0.2	14.3 ± 0.1	19.1	19.7 ± 0.3	64.3	27.0 ± 0.3	125.0

Table 3. Continued

In leaves (after third treatment)							
MDA, μM·g^{-1} fr.w.	32.4 ± 0.4	28.1 ± 0.3	-13.1	26.3 ± 0.5	-18.7	25.5 ± 0.4	-21.1
SOD, conv. un.·g^{-1} fr.w.	62.8 ± 0.7	66.9 ± 0.9	6.5	69.1 ± 0.7	10.1	78.4 ± 0.5	24.8
CAT, mM·g^{-1} fr.w.	1.4 ± 0.02	1.8 ± 0.01	28.6	1.6 ± 0.01	14.3	2.2 ± 0.04	49.2
APX, mM·g^{-1} fr.w.	8.4 ± 0.1	9.6 ± 0.2	14.1	11.4 ± 0.2	35.1	12.4 ± 0.4	47.6
GR, mM · g^{-1} fr.w.	125.4 ± 2.1	195.3 ± 4.3	55.7	196.1 ± 4.6	56.4	239.3± 2.1	90.8
GPX, mM·g^{-1} fr.w.	85.4 ± 1.9	106.6 ± 2.1	24.7	112.6 ± 2.5	31.8	217.0 ± 3.6	25.6
In bulbs (after third treatment)							
MDA, μM ·g^{-1} fr.w.	16.2 ± 0.2	15.2 ± 0.3	-7.2	14.2 ± 0.3	-12.0	12.1 ± 0.2	-25.15
SOD, conv. un. ·g^{-1} fr.w.	52.8 ± 0.8	57.9 ± 0.6	9.6	64.1 ± 0.4	21.4	70.1 ± 0.5	32.82
CAT, mM·g^{-1} fr.w.	2.1 ± 0.1	2.3 ± 0.1	8.4	2.9 ± 0.1	34.1	3.0 ± 0.1	39.25
APX, mM ·g^{-1} fr.w.	7.2 ±0.1	8.6 ± 0.1	19.7	9.1 ± 0.2	27.4	11.3 ± 0.02	5.96
GR, mM · g^{-1} fr.w.	182.8 ±2.9	205.3 ± 5.6	12.3	206.1 ± 4.7	12.7	227.0 ± 3.9	24.18
GPX, mM ·g^{-1} fr.w.	95.2 ± 2.3	101.3 ± 1.9	6.5	119.4 ± 1.7	25.5	120.2 ± 2.1	26.34

Compared to the control, plants treated with gibberellin contain in leaves in average 6.8 percent less malondialdehyde (MDA), considered as a marker of oxidative stress and peroxide oxidation of lipids; plants treated with potassium selenate had 12.5 percent less and Fludisec treated plants have 13.6 percent less content of MDA in leaves. Malondialdehyde is a product of peroxidation of unsaturated fatty acids from phospholipids and is responsible for degradation of cellular membranes. Thus, plants supplemented with selenium compounds display a lighter oxidative stress compared to the control plants. It is known that in continuously normal conditions in plant cells a certain level of lipid peroxide oxidation occurs, and it remains constant due to the antioxidant protection system. Probably, differences in lipid peroxide oxidation process intensity and in malondialdehyde formation detected in plants treated with physiologically active substances are due to different rates of activity of the enzymes of antioxidant protection. Plants treated with gibberellin, potassium selenate and, especially, with the solution of the coordination compound, are characterized by a significant increase in the activity of antioxidant protection enzymes: superoxide dismutase, catalase, ascorbate peroxidase, glutathione peroxidase, and other enzymes participating in the elimination of free radicals (Table 3).

In particular, the plants treated with Fludisec are characterized by the increased activity of superoxide dismutase (SOD), catalase (CAT), peroxidases (PX), GR etc. At the same time, Scandalios J.G.[22] mentions that CAT and APX are the most efficient enzymes for preventing cellular damage, by regulating H_2O_2 content, formed as a result of superoxide dismutase activity. But, besides CAT and APX, other peroxidases participate in hydrogen peroxide neutralization, these peroxidases can, according to the literature, be activated by selenium compounds. Glutathione peroxidase (GPX) was the first functionally characterized selenoprotein. Glutathione peroxidase reduces H_2O_2 and transforms hydroperoxides of lipids and phospholipids into harmless products (water and alcohols). The enzyme uses glutathione (GSH) as substrate and acts according to the reaction $ROOH + 2GSH \rightarrow R - OH + GSSG + H_2O$; in which: ROOH can be H_2O_2 or an organic peroxide, GSSG – glutathione disulfide (oxidized form of glutathione). As follows from the data in Table

3, in plants treated with Fludisec solutions, glutathione peroxidase activity in leaves increased 2.5-2.3 times compared to the leaves of the control plants. So, the results of the research demonstrate that the enzymatic antioxidant status of garlic pants treated with selenium compounds is higher than that of the control plants. Besides, the effect of oxidative destruction, ROS function as signaling molecules, inducing the reactions of defense and adaptation by activating or *de novo* synthesizing the components of the antioxidant system of protection, both antioxidant enzymes and non-enzymatic compounds, necessary for elimination of free radicals.[21, 26] Antioxidants with a small molecular weight modulate ROS concentration, maintaining it at a level necessary for their function as signaling molecules. ROS as signaling molecules activate the expression of genes, associated with the production of enzymes neutralizing oxygen radicals. Substances providing antioxidant protection include the compounds which, entering in the reaction with ROS, have a property to form either molecular products or radicals with a smaller reaction capacity. Plant foliar treatment with the solutions of selenium-containing compounds caused a significant increase in the content of Pro and ascorbate in the organs, compared to the gibberellin effect. The greatest effect was observed in case of application of the selenium coordination compound (Fludisec) (Table 4). Using gibberellin, potassium selenate and Fludisec as plant treatment caused the increase in proline concentration in leaves by 22%; 36% and 70%, respectively, and in bulbs by 6%; 17% and 29%, compared to the control (Table 4).

As it is known, proline has a number of useful functions during plant response to stress factors, including osmotic adjustment, stabilization of cell structures and cellular membranes.[27, 28] Under suboptimal environmental conditions, proline ensures the stabilization of protein molecules in cell membranes, the increase of the water retention capacity of these molecules; it can also serve as a respiratory substrate with repercussions on the energy potential of the cell; it participates in the elimination of free radicals.[27] Probably, the increase of the proline content in the plant organs treated with selenium compounds is a result of optimization of the assimilating pigments content (Table 2), since the carbohydrates produced during photosynthesis as well as glucose, fructose, mannitol, and, especially, sucrose, play an important role in biosynthesis and accumulation of Pro and ascorbic acid[28].

Ascorbate, together with glutathione, play a key role in maintaining intracellular redox equilibrium in plants.

Plants with a lower content of ascorbate are sensitive to ROS, generated by biotic or abiotic stress or senescence possibly due to a decrease in ROS detoxification. Ascorbic acid and glutathione, connected by the ascorbic acid cycle-glutathione reductase (GR), are essential for protection against oxidative damage.[29] In the present work, a significant influence of plant treatment with the solution of selenium compounds, especially with coordination compound Fludisec, on ascorbic acid assimilation in the organs of garlic plants was observed (Table 4).

Table 4. Proline content (μg · g^{-1} m. p., fr.w.) and ascorbate content (mg%) in the organs of *Allium sativum* L. plants treated with gibberellin and potassium selenate.

Organ	Control	*Gibberellin*		Potassium selenate		Fludisec	
	M±m	M±m	Δ, % contr.	M±m	Δ, % contr.	M±m	Δ, % contr.
			proline, μg · g^{-1} m. p.				
leaves	0.240 ± 0.01	0.293 ±0 .01	22.08	0.327 ±0.01	36.25	0.410 ±0.01	70.83
bulbs	1.735 ±0.04	1.850 ± 0.03	6.63	2.232 ± 0.05	28.65	2.247 ±0.05	29.51
			Ascorbic acid, mg%				
leaves	20.23±0.49	23.50±0.30	16.16	26.4±0.25	30.49	36.5±0.22	80.42
bulbs	7.48±0.15	7.80±0.12	4.28	8.8±0.18	17.65	10.2±0.20	36.36

Ascorbic acid possesses a wide spectrum of antioxidant properties, being donor and acceptor of hydrogen due to the presence of two enolic groups in the structure. It is the most efficient inhibitor of the oxygen singlet; H_2O_2 can be reduced both directly from ascorbate and by ascorbate peroxidase. Antioxidant properties of ascorbate are based on the cyclic transformations between dihydro-, semihidro- and dehydroascorbate forms. The reduced form has the property to directly interact with ROS and to reduce the α-tocopherol radical, glutathione (GSH), returning to them the properties of antioxidants. Ascorbic acid is the best protector from lipid peroxidation.

It should be mentioned that the total antioxidant status shows a significant increase in all plants treated with physiologically active substances – gibberellin, potassium selenate and, especially, with the solution of the coordination compound Fludisec. The increase of the antioxidant potential in plants supplemented with selenium is evidenced not only by a more intensive activity of the components of enzymatic system of antioxidant protection, but also by the increase of the number of compounds with small molecular weight involved in elimination of free radicals.

It is considered that intensification of antioxidant properties is due to the increase of the content of selenium in plant organs, which in turn is in linear correlation with its content in soil. Se absorption by plants depends on many factors, but when Se is present in a soluble form, it is rapidly absorbed by plants, even though differences between plant species are very pronounced. Most plants have low content of Se, below 25 μg/kg. Some mono- and dicotyledonous plant species are capable of absorbing Se by leave surface, and later transport and accumulate it in roots in different forms – from inorganic selenite to Se-based organic compounds. This postulate was also confirmed by the present work. Foliar treatment of garlic plants with gibberellin which enhances Se absorption from soil[16] and with solutions of potassium selenate and coordination compound Fludisec resulted in a moderate increase of Se content in leaves and bulbs of plants grown in regular conditions (Table 5).

The maximal increase of selenium content in leaves is observed in plants treated with potassium selenate, as well as in plants treated with Fludisec, whereas in the bulbs the maximal increase of selenium content is observed in case of Fludisec treatment (table 5). Selenium content in plants treated with potassium selenate and Fludisec solution is higher than that of the gibberellin-treated plants as well.

Table 5. Effect of garlic plant treatment on selenium content in leaves and bulbs (μg·kg^{-1} fr.w.)

Variants	**In leaves**		**In *bulbs***	
	M ± m	**Δ, %**	**M ± m**	**Δ, %**
Control	74.0± 1.8		47.0 ± 0.7	
Gibberellin	85.0 ± 2.1	14.86	59.0± 1.2	25.53
Potassium selenate	91.0 ±1.9	22.97	62.0 ±1.5	27.65
Fludisec	91.0 ±1.7	22.97	70.0±1.1	48.94

It is worth noting that in the leaves, regardless of the plant treatment, selenium content is about 21–27 μg · g^{-1} m.p. higher than in the *bulbs*. This allows suggesting that plants have a certain control mechanisms of selenium accumulation in leaves and bulbs. The obtained data support to some extend the suggestion made by Golubkina N.A. et al.[16] that plants have a hormonal mechanism of regulation of selenium content.

Kabata-Pendias A.[30] has mentioned that selenium deficiency in soils where crops are cultivated can cause not only a decrease in the content of this microelement in food products with negative consequences on human and animal health, but also a reduction of plant productivity. According to the data obtained by other research groups[31, 32], application of selenium in low concentrations can induce an increase of the yield of some crops. Some authors believe that selenium influence on crop productivity is determined by its participation in chloroplast formation, chlorophyll synthesis and intensification of photosynthesis[17]. Recent studies of some cereal crops and legumes produced some data that after application of Se, the growth rate of these plants can increase[4, 30]. The obtained results (Table 6) in the context of the present research demonstrate that the optimization of the functional state of plants by foliar treatment with gibberellin, and especially with potassium selenate stimulated growth processes and biomass accumulation of garlic plants.

Table 6. The effect of the physiologically active substances on productivity of garlic plants.

Variants	**Plant weight, g**		**Productivity g · pl.$^{-1}$**		**Yield, kg / parcel**	
	M ± m	Δ, %	M ± m	Δ, %	M ± m	Δ, %
Control	64.44±0.82		36.289±0.73		3.048±0.09	
Gibberellin	68.87±1.08	6.87	39.000±0.58	7.47	3.510±0.13	15.15
Potassium selenate	76.13±0.59	18.14	42.865±0.49	18.12	3.858±0.12	28,14
Fludisec	78.10±0.74	21.20	44.345±0.64	22.20	3.990±0.08	30.90

Biomass accumulation of the plants treated with gibberellin, potassium selenate and Fludisec is significantly higher than that of the control plants. The increase is by 6.87, 18.14 and 21.2 %, respectively. The average weight of the bulbs is, respectively, 7.47, 18.12 and 22.2 percent higher than that of the control.

So, bulbs treatment before planting and foliar treatment of plants during vegetation period with the solution of selenium-containing compounds ensures inhibition of membranes peroxidation and oxidative damage to the cells, by increasing the content of assimilating pigments, antioxidant compounds with small molecular weight, activity of the enzymatic system of protection from oxidative stress, with an impact on plant productivity. Protective action of selenium is evidenced by a decrease of the content of malondialdehyde, increase of the amount of chlorophyll pigments and carotenes, activation of superoxide dismutase, catalase, ascorbate peroxidase, glutathione peroxidase and glutathione reductase.

Experimental

The object of the research was garlic plants (*Allium sativum* L.), variety Izumrud, grown on experimental fields of the Institute of Genetics, Physiology and Plant Protection of the Academy of Sciences of Moldova. Plants are characterized by medium precocity, growth period until harvest 109-119 days, clove yield of 8.5-11.0 t/ha.

The research has been carried out in the experimental field with random allocations of variants in 3 blocks. Plant density was 30 plants per m^2. Parcel surface in each replicate was 3 m^2.

For pre-treatment of bulbs and treatment of plants during growth period, the recommendations of Golubkina N.A. (2015); Golubkina N.A., Papazian T.T. (2006) were followed with respect to the concentration of gibberellin and potassium selenate.[16, 33] Optimal concentrations of the solution of the new compound were determined during preliminary experiments.[9] In the field experiments, bulbs pre-treatment before planting and foliar treatments for 3 times during plant growth period with intervals of two weeks between treatments with respective solutions was carried out. The experimental scheme includes the following variants: 1. Control; 2 plants treated with gibberellin solution; 3. Plants treated with potassium selenate solution; 4. Plants treated with Fludisec solution in the optimal physiological concentration determined according with preliminary tests.

Analyses of the physiological processes were carried out on the leaves after each treatment and on the bulbs after the second and the third treatment of plants. The antioxidant status values were judged based on the changes in contents of malondialdehyde (MDA), and activities of superoxid dismutase (SOD), catalase (CAT), ascorbate peroxidase(APX), glutathione peroxidase (GPX) and glutathione reductase (GR). The intensity of peroxide oxidation of lipids (POL) was measured by determining the final product - malondialdehyde content.[34] The activity of key enzymes for antioxidant protection was determined by spectrophotometry: SOD – by the method described in[35]; CAT – following decomposition of H_2O_2 at λ 240 nm[36]; GPX – by oxidation of guaiacol (2-methoxyphenol) as hydrogen donor in the presence of H_2O_2 at λ 470 nm; APX – by monitoring the oxidation rate of ascorbate at λ 290 nm[37]; GR – by reducing glutathione oxidized in the presence of $NADPH+H^+$, λ 340 nm[38]; GPX – by oxidizing reduced glutathione, 260 nm[39]; content of assimilatory pigments by a spectrophotometric method.[40] Homogenization of plant material, liquid nitrogen fixation and extraction was done as described before.[41] The content of selenium in plant leaves and bulbs was measured after burning lyophilized samples in concentrated nitric acid followed by microwave extraction. The analysis was carried out using an atomic absorption spectrometer AAnalyst 800 (Perkin Elmer) with automatic injection of the sample, the volume of the injected sample was 20 microliters. 5 microliters of palladium ion containing matrix modifier was added to each sample by autosampler.[42, 43] Each sample was analyzed in three replicates.

Differences between the variants were documented and then subjected to ANOVA in Statistica 7 software.

As an additional source of selenium, a coordination compound tetrafluoroborate-[bis(dimetylglyoximato)-(selenocarbamide)$_{1.4}$-(selenium-selenocarbamide)$_{0.5}$-(selenium-selenium)$_{0.1}$cobalt(III)] with chemical formula $[Co(DmgH)_2(Seu)_{1.4}(Se\text{-}Seu)_{0.5}(Se\text{-}Se)_{0.1}][BF_4]$ was used, it includes three types of ligands with selenium: selenourea, selenium-selenourea and selenium-selenium, which would increase the assimilating capacity of this element by plants.

Crystal structure of the complex $[Co(DmgH)_2(Seu)_{1.4}(Se\text{-}Seu)_{0.5}(Se\text{-}Se)_{0.1}][BF_4]$ was determined by X-ray diffractometer Xcalibur equipped with the CCD detector at room temperature, and is shown in Figure 1. Crystallographic data indicate triclinic singony, spatial group of symmetry P-1, parameters of elementary cell a=7.9163(9), b=11.679(2), c=13.433(3) Å, α=64.50(2), β=75.31(1), γ=82.05(1)°, V=1083.8(3) $Å^3$, number of independent structural units Z=2, ρ(calc., g/cm^3)=1.906 for the composition $C_{9.9}H_{21.6}CoF_4N_{7.8}O_4Se_{2.6}B$. The structure was determined by direct methods, and the coordinates of basal atoms were detected by the least squares method in anisotropic variant using set of programs SHELX-97[44].

Conclusions

The elaborated procedure makes feasible quality improvement of garlic plants due to the increase of antioxidant properties of leaves and bulbs, as well as the content of active substances. The technical result of application of this procedure is the optimization of growth, to increase plant productivity, decrease lipids peroxidation, intensify enzymes activities of antioxidant protection system; protect assimilating pigments from oxidative damage, increase of the content of selenium in leaves and bulbs of garlic plants.

Treatment of plants with Fludisec solution is a promising method of increasing the antioxidant properties of garlic plants.

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