Advancement in Medicinal Plant Research
Vol. 4(3), pp. 99-105, August 2016
ISSN: 2354-2152
Full Length Research Paper

Agronomical biofortification of garlic plant (*Allium sativum* L.) in aspect of increasing selenium content and antioxidant properties

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Accepted 28 July, 2016

ABSTRACT

The paper presents results of studying the effect of garlic plant (*Allium sativum* L.) foliar treatment with gibberellins and potassium selenate on the yield, selenium content and antioxidant protection capacity of the leaves and bulbs. It has been found that biological fortification of the garlic plant by treating the leaves with the solution of potassium selenate increases the selenium content in leaves and bulbs, the capacity of the antioxidant protection of cells, the content of the proline, pigment assimilation and, reduces lipid peroxidation. Potassium selenate amplifies the adaptive potential of plants, reduces the negative impact of moisture deficit, enhances the accumulation of the substances that increase the water retention capacity in the leaves, and optimizes the processes of growth and productivity.

Keywords: *Allium sativum* L., antioxidant properties, selenium content, chlorophyll, carotenoids, productivity.

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INTRODUCTION

One of the important goals of modern agriculture is to obtain products with high content of microelements and vitamins for protection against reactive oxygen species that cause oxidative stress in the body of both, plants and animals, as well as people. Particular attention is given to products rich in selenium because of its importance in the alimentary chain. Currently, selenium has been identified as an essential mineral nutrient for the human body with an important physiological role in the prevention and cure of certain diseases. The effectiveness of anticarcinogenic compounds has recently been extensively investigated (Fordyce, 2005; Finley, 2005). The more active and useful has been considered in the organic form of selenium produced by the human organism from vegetable products. The content of selenium in foods, fruits and vegetables is dependent on the concentration in the soil (Щелкунов et al., 2000). Most of the world's soils are characterized by selenium deficiency. Exception is the western regions of Canada, USA, Columbia, Venezuela, Australia, Israel and Ireland. In Moldova, as well as in Romania, the soil is low in selenium and, therefore, it is possible that the daily requirement of the human organism is not covered (Kyrylyuk, 2006; Tom et al., 2006; Vlad-Ioan, 2011). It is also worth mentioning that fertilizers containing selenium have no noticeable effect due to nitrates, phosphates and chlorides, binding selenium in insoluble compounds. In black soils selenium is mostly in the form inaccessible to plants.

Reduction of selenium content in the human organism, revealed in some areas of the world, can be supplemented by dietary diversification, fortification of foods during processing. Another method would be biological fortification, increased levels of biologically available essential elements in the edible parts of plants grown using fertilizers or physiologically active substances (agronomic biological fortification) or by selection of plants with properties to concentrate selenium (genetic biological fortification). In this aspect there were highlighted in a number of plant species the accumulators of this trace element. Garlic (*Allium sativum*

L.) is able to accumulate such necessary trace elements including selenium, but the contents of the garlic bulbs from the soil depends on the content in the soil and its accessibility for plants. The research has shown that this element may be necessary for plants-accumulators and contribute to increasing crop yields.

The effect and mechanism of the selenium action is not being fully deciphered, although lately research in this aspect contributed more and more, they even have undertaken further work with this essential trace element in both plants and pharmaceutical preparations for them.

In our research, we have initiated an experimental study on: a) the effect of gibberellins and potassium selenate administered extra root, on the biological performance of garlic plants; b) deciphering the mechanisms whereby selenium as a trace element is involved in antioxidant protection induced by reactive oxygen species (ROS).

Experimental research objectives have been as follows: exploring and validating the method for enriching *Allium sativum* L. Plants with selenium; evaluating the potential of the garlic plant as the protective antioxidant after biological fortification with selenium.

MATERIALS AND METHODS

Garlic plant (*Allium sativum* L.) of Izumrud variety cultivated in the experimental fields of the Institute of Genetics, Physiology and Plant Protection of the Moldovan Academy of Sciences has served as the research object. The plants have been of average precocity, the vegetation period up to harvest has lasted from 109 to119 days, the harvest of bulbs has ranged from 8.5 to 11.0 t/ha. The research has been conducted in experimental plots with random assignment of variants in three blocks.

The experiments scheme comprised the following variants: 1- control; 2- plants pre-treated with the solution of gibberellins (125 mg.L^{-1}); 3- plants pre-treated with the solution of potassium selenate (40 µg Se·L^{-1}). Plant density has been 28 plants per sq. m. Area of the parcel is equal to 3 m^2 in each replicate. Pre-treatments of leaves with the appropriate solutions were carried out 3 times during the vegetation period with a break of two weeks. The analysis of physiological processes in the leaves was performed after each treatment and that of bulbs after the second and third processing. Status parameters of the water were determined by conventional methods (Vasseu and Sharkey, 1989; Kushnirenko et al*.*, 1970). The values of the total antioxidant status were evaluated by the degree of modification of the content of malonedialdehyde (MDA) and superoxide dismutase activity (SOD), catalase (CAT), ascorbate peroxidaze (APX), glutation peroxidaze (GP), glutation reductaze (GR). Testing of the intensity of lipid peroxide oxidation (LPO) was carried out with determination of the final product – the content of malonedialdehyde (Kurganova et al., 1997). The activity of key enzymes of antioxidant protection has been determined by the spectrophotometric method SOD (Chevari et al., 1985); CAT method (Chance and Machly, 1955) by spectrophotometric determination at 240 nm λ decomposition of H_2O_2; GwPX - after oxidation intensity guaiacol (2 - methoxy - phenol) as a hydrogen donor in the presence of H_2O_2 at λ 470 nm; APX - by monitoring the rate of oxidation of ascorbate at λ 290 nm (Nacano and Asada, 1981); GR - by reduction of oxidized glutathione in the presence of NADH · H2, λ 340 nm (Schadle and Bassham, 1977); GPX - by oxidation of reduced glutathione, 260 nm (Woodland et al., 2004); assimilating pigments content - spectrophotometric method (Ermakova et al., 1987). Homogenization of the plant material, fixed in liquid nitrogen, and extraction - as described (Keshavkant and Naithani, 2010).

The content of selenium in plant leaves and garlic bulbs was determined after the firing of the freeze-dried samples in concentrated nitric acid and subjected to microwave assisted extraction. The analysis was performed with atomic absorption spectrometer Analyst 800 (manufactured by Perkin Elmer) with automatic sample injection; the sample volume injected was 20 µl. Added to each sample was auto-sampler and 5 µl and the matrix modifier, which consists of palladium ions (Matek et al., 1999; Izgi et al., 2006). Each sample was analyzed in triplicate.

Differences between the versions have been documented by statistical analysis using the arithmetic mean error and Student's test significance.

RESULTS AND DISCUSSION

In recent years data have appeared in literature showing that selenium in small concentrations conditions amplification of adaptive potential of plants, diminishes the negative impact of droughts, stabilizes the surface of the assimilation apparatus, reduces the fall of flower buds and contributes to the activation of growth processes in the recovery period after improving moisture conditions (Seregina, 2008; Skrypnyk, 2009). A selenium deficit inhibits plant growth, inhibits flowering, and results in losing tolerance to changing factors of the environment, chlorosis, loss of turgor and wilting.

The present study results show that plants treated with gibberellins and those treated with potassium selenate demonstrate a tendency of increasing values of the water status (Table 1). Treatment of leaves with gibberellins increases the hydration of the leaves by 2.08% and bulbs by 1.42% as compared with control plants, decreases the saturation deficit and increases tissue turgor, respectively, by 11.5 and 18.66%, the differences being statistically accurate after I and II degree of truthfulness (ß = 3.66 to 6.44). In plants receiving potassium selenate the water content in leaves and bulbs exceeded the value of this index to control plants by 2.47 and 2.14% accordingly; while the value of saturation deficit was lower compared with control plants by 16.65% in leaves and by 22.39% in bulbs. The plants pre-treated with potassium selenate demonstrated a stabilizing effect of water status significantly higher than plants pre-treated with gibberellins (Table 1). The persistence of hydration and turgidity of plant leaves is due to increase of their capacity to retain water in tissues.

Therefore, the results of the study of modification in water status of plants confirm the idea that selenium may reduce the negative impact of drought.

It is known that impact of unfavourable factors, including drought and tissue dehydration conditions, excessive formation of reactive oxygen species (ROS) and the emergence in cells affects the status of oxidative stress (OS). Some recent works (Xue and Hartikainen, 2000; Seppänen et al., 2003; Kabata-Pendias, 2011)

Table 1. The effect of applying the compounds on water status in the garlic plant leaves and bulbs.

Variants	WC*, g/100 g f.w.		DS*, % to full saturation		WRC*			
					% lost water		% remaining water	
	M ± m	Δ, %	M ± m	Δ, %	M ± m	Δ, %	M ± m	Δ, %
	In leaves (after the II-nd pre-treatment)							
Control	81.67 ± 0.27		9.07 ± 0.10		2.95 ± 0.07		79.26 ± 0.17	
Gibberellin	83.37 ± 0.10	2.08	8.03 ± 0.13	-11,47	2.98 ± 0.06	1.02	80.89 ± 0.08	2.06
Potassium selenate	83.81 ± 0.17	2.62	7.56 ± 0.22	-16.65	2.77 ± 0.05	-6.10	81.49 ± 0.11	2.81
	In bulbs (at harvest)							
Control	56.36 ± 0.20		1.34 ± 0.05		1.61 ± 0.03		55.45 ± 0.14	
Gibberellin	57.16 ± 0.15	1.42	1.09 ± 0.03	-18.66	1.47 ± 0.04	-8.70	56.32 ± 0.07	1.57
Potassium selenate	57.75 ± 0.27	2.47	1.04 ± 0.01	-22.39	1.38 ± 0.02	-14.29	56.95 ± 0.12	2.70

*- WC - water content; DS – deficit of saturation; WRC - water retention capacity.

stated that Se is the antioxidant that activates protective mechanisms and reduces the oxidative stress in plant chloroplasts under UV stress. According to data obtained in this study, supplementation with the selenate of garlic plants (*Allium sativum* L.) reduces the impact of the oxidative stress caused by drought (Table 2).

In multiple studies conducted previously it has been shown that dehydration of organs induced by drought is the direct sequence of stomatal closure, disorganization of photosynthesis, inhibition of mechanisms of antioxidant protection, increased production of reactive oxygen species, affecting normal homeostasis of the organism.

Excessive formation of ROS in unfavourable conditions, lipid per oxidation, protein oxidation, degradation of nucleic acids, enzyme inhibition, activated the programmed cell death (Neill et al., 2002).

In cases when ROS concentration reaches its threshold, intensification occurs by oxidation of lipid peroxidation (LPO) in all cell membranes, which affects the normal functioning of cells. LPO aggravates the oxidative stress by the fact that the production of radicals derived from LPO can react with proteins and DNA (Sharma and Dubey, 2005; Tanoue et al., 2009). Saturated fatty acids are very sensitive to ROS attacks as a single $^{\bullet}OH$ can peroxidize more polyunsaturated acids; the chain disruptions cause reactions in the structure and metabolic processes. Saturated fatty acids are very sensitive to ROS attacks as a single $^{\bullet}OH$ can peroxidize more polyunsaturated acids, being the reason of chain disruptions in the structure and metabolic processes. Taking into account the level of MDA (Table 2), it can be concluded that ROS production in garlic plant organs treated with gibberellins, and, especially, with potassium selenate, is actually much lower as compared to control plants. Due to high MAD content the leaves and bulbs characterize, control plants, which together account for about 32.35 ± 0.38 and 16.18 ± 0.20 mkM. g.f.w. In the plants which have been treated with gibberellins, the MAD content decreased by 13.08 and 7.18%, respectively. Under the influence of potassium selenate MAD content has been reduced by 18.73% in leaves and by 11.99% in bulbs (Table 2). From the results of the investigation, it is followed that the dehydration of tissues and formation of ROS caused significant oxidative destruction in control plant's leaves and bulbs as compared to plants pre-treated with potassium selenate. The control plants are characterized by a lower antioxidant protection capacity than plants supplemented with selenate.

Under optimal permanent conditions in plant cells there is a certain level of lipid per oxidation, which remains constant due to antioxidant protection systems. An essential role in protecting cells from oxidative destruction belongs to enzyme system, in particular, superoxide dismutase, which catalyzes the reaction of hiding superoxide radicals ($O^{\cdot -}$). Speed of interaction of SOD and $O^{\cdot}$ depends on both viscosity of membranes and the degree of hydration of cells (Asada, 2006). Depending on the intensity of drought, SOD activity changes differently - at a moderate drought in enzyme activity, intensifies

Table 2. Malondialdehyde content and antioxidant protective enzyme activities in leaves and bulbs of garlic plant (*Allium sativum* L.).

Parameters	Control	Gibberellin		Potassium selenate	
	M ± m	**M ± m**	**Δ,%**	**M ± m**	**Δ,%**
In leaves (after the III-rd pre-treatment)					
MDA, мkmol · g^{-1} f.w.	32.35 ± 0.38	28.12 ± 0.29	-13.08	26.29 ± 0.45	-18.73
SOD, conv.units · g^{-1} f.w.	62.81 ± 0.73	66.86 ± 0.92	6.45	69.14 ± 0.68	10.08
CAT, mmol · g^{-1} f.w.	1.30 ± 0.015	1.78 ± 0.009	36.92	1.61 ± 0.011	23.85
APX, mmol · g^{-1} f.w.	8.43 ± 0.13	9.62 ± 0.17	14.12	11.39 ± 0.15	35.11
GlR, mmol · g^{-1} f.w.	172.8 ± 2.08	195.31 ± 4.27	13.00	196.09 ± 4.63	13.45
GlPX, mmol · g^{-1} f.w.	85.44 ± 1.94	106.58 ± 2.15	24.74	112.59 ± 2.47	31.78
In bulbs (after the III-rd pre-treatment)					
MDA, мkmol · g^{-1} f.w.	16.18 ± 0,20	15.18 ± 0.34	-7.18	14.24 ± 0.28	-11.99
SOD, conv.units · g^{-1} f.w.	52.81 ± 0.77	57.86 ± 0.61	9.56	64.13 ± 0.43	21.44
CAT, mmol · g^{-1} f.w.	2.14 ± 0.012	2.32 ± 0.07	8.41	2.87 ± 0.9	34.11
APX, mmol · g^{-1} f.w.	7.16 ± 0.10	8.57 ± 0.14	19.69	9.12 ± 0.16	27.37
GlR, mmol · g^{-1} f.w.	182.84 ± 2.95	205.31 ± 5.62	12.29	206.09 ± 4.73	12.72
GlPX, mmol · g^{-1} f.w.	95.15 ± 2.31	101.34 ± 1.87	6.51	119.43 ± 1.68	25.52

while in lengthy drought enzyme activity decreases (Ştefîrţă et al., 2014, 2015). The experimental results shown in Table 2 confirm those reported. Under drought conditions of summer 2015 on plants treated with gibberellins and with potassium selenate, there was recorded SOD activity increase in leaves by 10.08 and 6.45%; and in bulb by 9.56 and 21.44% in comparison with the enzyme activity in the control plants organs. The potassium selenate administration has ensured a better mitigation of the drought impact that manifested an actual enhancing activity of enzymes with the antioxidant protection. The trend of SOD, CAT, APX, GPX, GR activity had tended to increase in leaves and bulbs (Table 2).

The rapid growth of SOD activity generates increasing of H_2O_2, the one that still leads to inhibition of the enzyme, which may cause destructions and higher issues because hydrogen peroxide has the ability to penetrate cell membranes and cause destructions away from the place of occurrence. The effective functioning of SOD is largely determined by the operation of other components of the system of antioxidant protection, particularly those that removes hydrogen peroxide (catalase, peroxidases), and enzymes with cycle ascorbic acid – glutathione. The data obtained have demonstrated that the activity of both enzymes has increased under the influence of gibberellins and potassium selenate (Table 2). It is worth mentioning that plants pre-treated with gibberellins have demonstrated a stronger activation of CAT and those pre-treated with potassium selenate demonstrated a more significant increase of APX. Similar results have been obtained when determining the change of GPX and GR activity, which has shown different role of enzymes in the degradation of hydrogen peroxide and maintaining the level of H_2O_2.

The nature of changing the content of MDA and activity of enzymes with antioxidant protection primarily evidences the influence of gibberellins and potassium selenate in processes of reducing the oxidative stress caused by drought. The antioxidant action of selenium is manifested by a tendency of normalization of these parameters; DAM values decreased significantly, not only to those of the untreated plant, and compared with the effect of gibberellins.

The sequence of the oxidative stress caused by drought is abundant generation singlet oxygen, destruction of chloroplasts and the drop of the content of pigments assimilation in leaves. According to data obtained in this study, filling garlic plants with Se by treating the leaves with potassium selenate have led to an increase in the content of assimilating pigments (Table 3). The plants pre-treated with a solution of gibberellins contained chlorophyll in leaves by 17.61%, higher than in control plant leaves and plants treated with the selenate contained green pigments by 2.6% higher. The plants pre-treated with the solution of gibberellins were characterized by the content of chlorophyll in leaves by 17.61% higher than in control plant leaves while the plants treated with the selenate contained more green pigments by 32.6%.

Attention has been drawn to the fact that the plants pre-treated with potassium selenate have actually increased the content of carotenoids, which have the function of protective green pigments from oxidative destruction. The impact of the potassium selenate on the content of assimilatory pigments is due to the fact that Se participates in the reactions of chlorophyll synthesis, in the synthesis of tricarboxylic acids, as well as fatty acid

Table 3. The impact of applying compounds with the selenate on the content of pigments in garlic plant leaves.

Variants	Chlorophyll a, mg/100 g f.w.		Chlorophyll b, mg/100 g f.w.		Chlorophyll a+b, mg/100 g f.w.		Carotenoids, mg/100 g f.w.	
	M ± m	Δ, %	M ± m	Δ, %	M ± m	Δ, %	M ± m	Δ, %
Control	20.21 ± 0.51		9.49 ± 0.16		29.70 ± 0.66		8.76 ± 0.20	
Gibberellin	23.81 ± 0.62	17.81	11.11 ± 0.28	17.07	34.93 ± 0.81	17.61	10.13 ± 0.22	15.64
Potassium selenate	27.03 ± 0.64	33.75	12.34 ± 0.2	30.03	39.38 ± 0.85	32.59	11.31 ± 0.28	29.11

Table 4. Proline (μkg · g^{-1}. f.w.) content in organs of plants *Allium sativum* L. pre-treated with gibberellins and potassium selenate.

Organ	Control	Gibberellin		Potassium selenate	
	M ± m	M ± m	∆, % Control	M ± m	∆, % Control
In leaves	0.240 ± 0.006	0.293 ± 0.008	22.08	0.327 ± 0.007	36.25
In bulbs	1.735 ± 0.047	1.850 ± 0.038	6.63	2.232 ± 0.047	28.65

metabolism with high molecular weight (Torshin et al., 1996).

The protective action of the selenium is manifested also by controlling the content of proline (Pro), which, as is known, has multiple beneficial functions in the response of plants to the impact of stress factors, including the osmotic adjustment, stabilization of cell structure, membranes, and elimination of free radicals (Szabados and Saoure, 2009). In dry conditions proline ensures stabilization of protein molecules in the cell membranes, increases water retention capacity by these molecules, can serve as the respiratory substrate with repercussion on the energy potential of the cell. The results of investigations have revealed the effect of the selenium in the leaves and bulbs of *Allium sativum* L. (Table 4).

Pre-treatment of plant foliar by the solution of potassium has resulted in a more significant increase of the proline content in organs compared to the effect of gibberellins. So far it is unclear which mechanisms of proline content increase are directly related to the action of selenium. It is assumed that the increase of the proline content is directly related to the accumulation of carbohydrates as a result of optimizing the content of assimilating pigments and photosynthesis, because it is known that carbohydrates such as glucose, fructose, mannitol, and, in particular, saccharose, play a significant role in the accumulation of proline (Kishor et al., 2005). We believe that the accumulation of proline under stress can result from de novo synthesis or its low degradation or impairment of transporting them from organ to another, or be the result of all these changes. Unstable nature of malondialdehyde content and activities of enzymes with antioxidant protection in treating plants confirms the mitigating effect of oxidative stress. The antioxidant action of selenium is manifested by a tendency to normalization of these parameters; MDA values decreased significantly, check plants and as compared to the effect of gibberellins.

It is considered that intensification of antioxidant properties is due to increase of selenium content in plant organs, which in its turn is in positive linear correlation with its content in the soil. The absorption of selenium by plants depends on many factors, but when selenium is present in soluble form, it is quickly absorbed by plants, although the differences between plant species are very pronounced. In this sense, the plants have been split into three categories:

1- accumulating plants (from 0.05 to 0.25 mg/kg); 2- plants absorbing the average amount up to about 100 pg of selenium / kg, and 3- plants that are not accumulators, that contain less than 30 pg/kg, under field conditions (Kabata-Pendias, 2011). The authors also have noted that the safety margin of the selenium average concentration in plants is quite narrow. Most plants have a low content of selenium, less than 25 mg/kg.

Some mono- and dicotyledonous plant species have the capacity to absorb the selenium through the leaf surface which is then is transported and accumulated in the roots in various forms - for selenite inorganic and organic compounds. This postulate has been confirmed in these investigations. Foliar treatment of garlic plants with gibberellins, which enhances absorption of seleniun from the soil (Golubkina et al., 2015), and with the potassium selenate resulted in the increase of selenium content in the leaves and bulbs of plants (Table 5). The content of selenium in the leaves and bulbs of control plants constitutes respectively the increase by 24.0 and 23.0 mg·kg of dry substance. After selenium content, the plants supplemented with potassium selenate have prevailed over the plants treated with gibberellins.

Some authors are of the opinion that selenium, participating in chloroplast formation and synthesis of chlorophyll influence on the intensity of photosynthesis, thus ensuring better productivity (Torshin et al., 1994;

Table 5. The impact of garlic plant treatments of selenium content in leaves and bulbs (µkg.kg^{-1} s.u.)

Organ	Control	Gibberellin 125 mg·L^{-1}		Potassium selenate 0.00001%	
	M ± m	M±m	Δ, % Check	M±m	Δ, % Check
In leaves	74.0± 1,8	84.0 ± 2.1	13.51	88.0 ±1,9	18.92
In bulbs	47.0 ± 0.7	59.0 ± 1.2	25.53	70.0±1,1	48.94

Table 6. The effect of gibberlins and potassium selenate on garlic plant productivity.

Variants	Plant weight, g·pl^{-1}		Plant productivity, g·pl^{-1}		Yield, kg/m^2	
	M ± m	Δ, %	M ± m	Δ, %	M ± m	Δ, %
Control	64.44 ±0.82		36.289 ± 0.73		2.177 ± 0.09	
Gibberellin	68.87±1.08	6.87	39.000 ± 0.58	7.47	2.340 ± 0.13	12.75
Potassium selenate	76.13 ±0.59	18.14	42.865 ± 0.49	18.12	2.575 ± 0.12	19.09

Swirling and Lebedev, 2010). Recent studies with some grass and vegetable crops have provided data that one adds of selenium may increase the growth rate of these plants (Kabata-Pendias, 2011). The results obtained during this study (Table 6) have demonstrated that optimization of the functional state of plants by foliar pre-treatment with gibberellins and especially with the potassium selenate has conditioned stimulation of growth processes and accumulation of garlic plant biomass.

Accumulation of pre-treated plant biomass accumulation gibberellins and potassium is actually higher than in the variant with control plants. The effect of the selenium increase is 18.14 and 6.87%, respectively. The average mass of bulbs prevails over the control by 7.47 and 18.12%, respectively. Therefore, we can conclude that the deficiency of selenium in the soil, which leads not only to decrease plant productivity, but also the inadequacy of selenium in animal and human body, can be prevented by treating the surface with plant foliar physiologically active substances or soluble compounds containing selenium. Garlic plant (*Allium sativum* L.) during the pre-treatment actively accumulates selenium, that is linked to the presence in cells of glycosides containing and sulphurum and which can be substituted with selenium atom with similar properties. Supplementing plants with selenium results in optimizing the implementation of adaptive potential of plants, which is manifested in enhancing antioxidant properties, increase of photosynthetic pigments fund, accumulation of biomass and plant productivity. Garlic plant leaves and bulbs supplemented with selenium are characterized by increased antioxidant properties.

CONCLUSIONS

1. Biofortification of garlic plants through treatment of the foliar apparatus with a potassium selenate solution provides an increase of the selenium content in leaves and bulbs, the antioxidant capacity of cells, proline content, assimilating pigments, and the reduction of lipid per oxidation.
2. The antioxidant impact of selenium manifested itself by a tendency to optimize these parameters; DAM values decreased significantly as compared to those of untreated plants.
3. Potassium selenate increases the adaptive potential of plants, reduces the negative action of moisture deficit, enhances the accumulation of the substances that increase the water retention capacity of the leaves and optimizes the processes of growth and productivity.

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Citation: Stefîrţă A, Botnari V, Brânză V, Bulhac I, Chilinciuc AL, 2016. Agronomical biofortification of garlic plant (*Allium sativum* L.) in aspect of increasing selenium content and antioxidant properties. Adv Med Plant Res, 4(3): 99-105.