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### RESEARCH ARTICLE

#### EXOGENOUS OPTIMIZATION OF THE ANTIOXIDANT PROTECTION CAPACITY OF PLANTS IN MODERATE DROUGHT CONDITIONS

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#### Abstract

The effect of Thiourea, Galmet and Thiogalmet on the antioxidant protection capacity of plants in moderate drought conditions was studied. Thiogalmet is a new chemical composition that contains Thiourea (Tu) and Galmet - a preparation that consists of a mixture of potassium, ammonium, magnesium gallates, and potassium molybdate and ammonium paramolybdate. Biological tests were performed in the vegetation complex of the Institute of Genetics, Physiology and Plant Protection. As subjects of investigations served the plants *Glycine max* (Merr) L 'Nadejda' variety and *Zea mays* L 'P458' cultivar, grown in the Mitcherli vegetation pots with 40 kg soil and exposed to the drought stress at the critical stages for water. It has been established that pre-treatment of plants with Thiourea, Galmet and, in particular, with Thiogalmet increases the activity of superoxide dismutase (SOD), catalase (CAT), ascorbateperoxidase (APX), glutathione reductase (GLR) and glutathione peroxidase (GLPX). The high activity of antioxidant enzymes is associated with a decrease in the content of malonic di-aldehyde (MDA). The increase in antioxidant protection capacity of plants pre-treated with Thiogalmet was recorded both in optimal conditions and in conditions of oxidative stress induced by drought. Thiogalmet has been shown to be one of the new biologically active chemicals that can be used in agriculture to reduce the negative impact of oxidative stress caused by reactive oxygen species (ROS).

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#### Introduction:-

Global warming, the phenomenon of a constant increase in average air temperatures and a decrease in precipitation, is one of the most difficult problems facing humanity today. Agriculture is one of the sectors most affected by these changes due to its dependence on meteorological conditions, which at the moment cannot be controlled. The complex effects of climate change cause significant qualitative and quantitative annual crop losses and in severe cases can jeopardize the whole yield. Thus, mitigating the negative impact of extreme natural phenomena and ensuring crop stability is one of the priorities of agriculture. [IPCC, 2011; Jentsch A., Kreyling J., Elmer M., Gellesch E., et al., 2011; Krasensky H., Jonak C., 2012]. A special attention is paid to developing innovative methods for exogenously regulating plant resilience and productivity, based on knowledge of the mechanisms involved in plant response and tolerance to ecological stress in countries with advanced agricultural technologies. It has become an axiom that under stress conditions there is an eruption of reactive oxygen species formation in plant

cells, which conditions, depending on their concentration, a different degree of oxidative destruction of cell structures. It is well argued [Bhattacharjee S., 2005, 2012; Mittler R., Blumwald E., 2010; Munne-Bosch S., 2013; Walter J., et al., 2013] that the key mechanisms associated with an adequate response to adverse humidity fluctuations and drought conditions are those associated with the autoregulation of ROSs formation and neutralization through activation or a more complete realization of the antioxidant potential. Plant tolerance to oxidative stress is associated with the activity of antioxidant protective enzymes and high levels of antioxidants in cells [Foyer, Shigeoka, 2011; Ștefîrță A. et al., 2012; 2017; S. Munne-Bosch et al., 2013; Bartolia et al., 2013]. The main antioxidants in higher plants include glutathione, ascorbate, tocopherol, proline, betaine and others, which are also important components of signaling pathways. Enzymatic and non-enzymatic antioxidants in higher plants can protect cells from oxidative damage by eliminating ROSs. In addition to their crucial role in the defense system and as enzymatic cofactors, antioxidants influence the growth and development of plants by modifying the processes of mitosis and cell elongation. The concentration of native protective substances is another important factor that determines cell and tissue tolerance to adverse conditions. The high level of substances with thiol-groups, the presence of antioxidants, free radical acceptors, and inhibitors of chain peroxide reactions decreases the sensitivity to drought due to free radical scavenging, inhibition of lipid peroxidation in membranes. In general, tolerant plants have a higher level of anti-stress metabolites under normal growing conditions and / or accumulate several metabolites with protective function such as amino acids, soluble carbohydrates, various antioxidants and others under unfavorable conditions. In this sense, the use of physiologically active substances (PHAS) with antioxidant properties presents a possibility of increasing crop productivity and quality under unfavorable conditions. [Asada K. 2006; Paris Sharifi, et al., 2012; Merewitz E., et al., 2015; Ștefîrță et al., 2015, 2021]. An impressive number of studies provide evidence of the effective role of Thiourea in modulating physiological mechanisms and improving plant productivity under both optimal and moderately unfavourable conditions [Wahid, A., S.M.A. Basra, M. Farooq, 2017]. Thiourea is a nitrogen and sulfur-containing compound that has amino-, imino- and thiol- functional groups, each with important biological roles. The main functional groups in the Thiourea molecule are the thiol (-SH) and amino (-NH<sub>2</sub>) groups, which make it one of the most important molecules in physiological processes. The thiol group helps stabilizing the structure of proteins. Moreover, the thiol group can capture ROSs in living cells. It is believed that Thiourea may be a nutritional supplement or may act as an agent for capturing reactive oxygen species (Pandey S., et al., 2013; Perveen A., et al., 2015).

On the other hand, the possibility of optimizing plant growth, development and enhanced productivity under conditions of moderate moisture insufficiency is known by treating seed material and foliar apparatus during vegetation with aqueous solution of Galmet - a preparation, containing K<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, Mg<sup>2+</sup> gallates and potassium molybdate and ammonium paramolybdate salts, necessary for plant normal growth and development [Ștefîrță A., Brînză L., Toma S., et al., 2008; Coropceanu E., Ciloci, A., Ștefîrță A., Bulhac, I., 2020]. Gallic acid, widespread in many plant species, is known to have strong antioxidant properties and free radical scavenging activity and can protect cells, tissues and organs from oxidative stress damage, form of free acids, esters, catechin derivatives and hydrolysable tannins. The antioxidant properties of gallic acid and its derivatives have been widely studied with tangents to their use for pharmaceutical purposes. Gallic acid and its derivatives are considered to be a group of natural polyphenolic antioxidants that have recently been shown to have potential healing effects [Singh Manish Pal, Gupta Avneet, Sisodia S Siddhraj. 2018; Bharti Badhani, Neha Sharma and Rita Kakkar, 2021]. Zhongbing Lu, Guangjun Nie, Peter S. et al. (2006) have shown that gallic acid derivatives with high antioxidant activity are generally more effective in preventing oxidative stress injury caused by neurodegenerative diseases.

It is scientifically documented that both Thiourea and Galmet have a positive influence on plant growth under both optimal humidity conditions and long-term drought. Both preparations adjust the optimization of tissue hydration degree, increase leaf turgidity, significantly intensify the assimilation of carbon dioxide and increase the efficiency of water use by plants [Coropceanu E., Ciloci, A., Ștefîrță A., Bulhac, I., 2020; Ștefîrță A., Bulhac I., Coropceanu E., Voloșciuc L., Brînză L., 2021]. Consequently, pre-treated plants are distinguished by a better formation of the assimilation surface, biomass accumulation, formation of the elements of productivity, as well as harvest. However, it should be noted that scientific sources lack the information on the antioxidant effect of Galmet on antioxidant plant protection systems.

Based on the abovementioned, one of the specific objectives of this study was to evaluate the antioxidant properties of Thiourea, Galmet and Thiogalmet and the effect of exogenous use of these preparations on non-specific mechanisms, correlated with the degree of manifestation of plant potential resistance to drought-caused oxidative stress. Tiogalmet is a new preparation containing Thiourea and Galmet.

## Materials and Methods:-

Plants of *Zea mays* L., 'P 458' cultivar and *Glycine max* (L.)Merr., 'Nadejda' variety, grown in Mitcherlich containers with 40 kg soil, were used as study objects. The experiments and the investigations were carried out in the Vegetation Complex and laboratories of the Institute of Genetics, Physiology and Plant Protection.

### The experiments were performed according to the following scheme:

Series I - plants grown from water-treated seeds (control);

Series II - seed material and plants treated during vegetative growth with aqueous solution of Thiourea;

Series III - seed material and plants treated during vegetative growth with aqueous solution of Galmet in a concentration of 0.005%;

Series IV - seed material and plants treated during vegetative growth with aqueous solution of Thiogalmet of the same concentration. On parallel variants, the effect of pre-treating the plants with the aqueous solutions of the compounds indicated above on the particularities of antioxidant protection of the plants in conditions of oxidative stress, created by reducing soil moisture, was studied. Drought conditions were created during the critical periods for plant: in maize - during panic - flowering; in soybeans - during flowering - the beginning of pod formation. Duration of water stress was of 7 days, followed by a recovery period.

The effect of pre-treatment of maize plants with Thiourea, Galmet and Thiogalmet on the indices characterizing the intensity of oxidative destruction (malonic di-aldehyde (MDA)) content and the activity of antioxidant protection enzymes (SOD, CAT, APX, GLPX, GLR), in the leaves of plants were studied.

SOD activity was determined by inhibiting the photochemical reduction of nitroblutetrazolium, described in detail by Becana M., Aparicio-Tejo P.M., Irigoyen J.J., Sanchez-Diaz M. (1986). The incubation medium contains K-Na-phosphate buffer (60 mM, pH 7.8), methionine (13 mM), riboflavin (2  $\mu$ M), nitroblutetrazolium (63  $\mu$ M), EDTA (0.1 mM) and 100  $\mu$ L the extract. The reaction takes 10 minutes at the light intensity of 15W fluorescent lamps. The samples incubated in the dark were used as controls. The enzyme activity that inhibits 50% of the photo-reduction of nitroblutetrazolium was taken for a conventional unit of SOD activity. CAT activity was estimated by the method of Chance B. and Machly A. (1955) by spectrophotometric determination at  $\lambda$  240 nm of H<sub>2</sub>O<sub>2</sub> decomposition; APX - by monitoring the oxidation rate of ascorbate at  $\lambda$  290 nm [Nacano Y., Asada K., 1981]; GLR - by reducing oxidized glutathione in the presence of NADP·H [Schadle M., Bassham J.A., 1977]. Plant material homogenization and extraction were performed as described in [Keshavkant S., Naithani S.C., 2001]. The activity of antioxidant ferments was expressed in mM of oxidized substrate and was appreciated as a percentage of the activity of ferments in the leaves of control plants. The protocol of the experimental results was documented in paged registers, stamped and approved by the administration of the institute. The results were statistically analysed, using the software package "Statistics 7" for PC.

Thiogalmet preparation, obtained in the Institute of Chemistry of the Republic of Moldova, was used in this study. It is a solid light-brown powder, stable at room temperature, well soluble in water. It was obtained by mixing two mass parts of Thiourea with one mass part of Galmet, which consists of potassium-, ammonium- and magnesium gallates, potassium molybdate and ammonium paramolybdate, taken in a mass ratio of 1:1:1:0.1:0.1 respectively. Bioactive component content in Thiogalmet, %: Thiourea - 66.66; gallate-anion ((OH)<sub>3</sub>C<sub>6</sub>H<sub>2</sub>COO<sup>-</sup>) - 21.52; K<sup>+</sup> - 1.63; Mg<sup>2+</sup> - 0.97; Mo(VI) - 0.99; NH<sub>4</sub><sup>+</sup> - 1.07; total nitrogen - 25.36. The IR spectrum of Thiogalmet composition was recorded on a FTIR Spectrum-100 Perkin Elmer spectrometer in Nuiol (400-700 cm<sup>-1</sup>) and using ATR technique (700-4000 cm<sup>-1</sup>).

## Results And Discussion:-

The IR spectrum of Thiogalmet (Fig. 1) is intricate due to the complexity of the preparations' chemical composition. It should be noted that the IR spectrum of Thiogalmet does not represent a simple sum of the absorption bands of Thiourea and Galmet components, which is explained by the fact that some processes such as association of functional groups, -NH<sub>2</sub>, -OH, NH<sub>4</sub><sup>+</sup> and others [Bellamy LJ, 1954] take place during homogenization of the components. However, some more intensive absorption bands specific for Thiourea and Galmet components can be identified. As follows, absorption bands of 3364 and 3271 cm<sup>-1</sup> are manifested for Thiourea, which is attributed to  $\nu_{as}(\text{NH}_2)$  and  $\nu_s(\text{NH}_2)$  oscillations respectively, associated via formation of hydrogen bonds, due to which they are shifted to the lower frequency region [Bellamy L. J., 1954; Исаков X, Аскарпов И.П., Усманов С., 2018], and the oscillation band  $\delta(\text{NH}_2)$  - 1609 cm<sup>-1</sup> for the same reason moves inversely towards the region of higher frequencies

[Bellamy L. J., 1954]. The absorption band of medium intensity at  $1050\text{ cm}^{-1}$  is attributed to the  $\nu(\text{C-N})$  oscillations in Thiourea molecules [Исаков Х, Аскарлов И.Р., Усманов С., 2018].

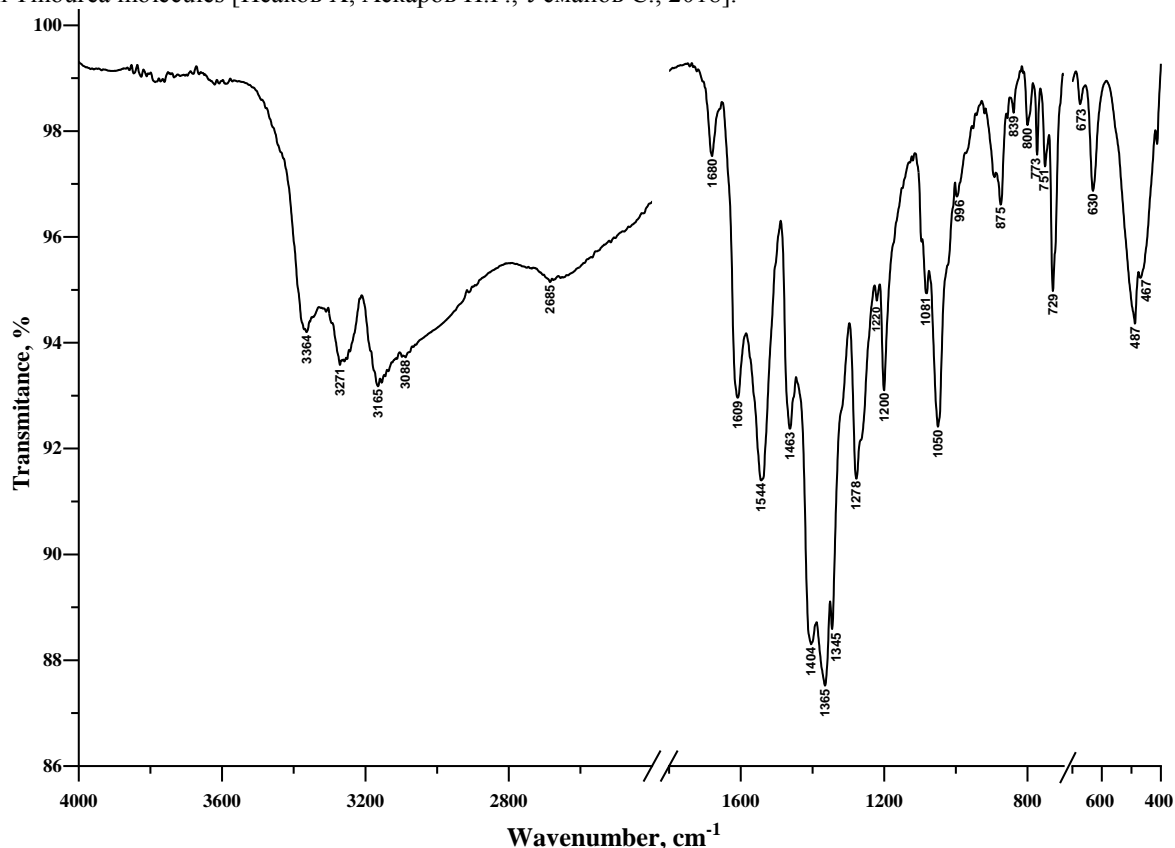


Fig. 1:- Thiogalmet IR spectrum.

The most intensive spectrum absorption band at  $1365\text{ cm}^{-1}$  is attributed to  $\nu(\text{C=S})$  oscillations [Bellamy L. J., 1954], and the average intensity band at  $487\text{ cm}^{-1}$  - to  $\delta(\text{NC=S})$  oscillations (Исаков Х, Аскарлов И.Р., Усманов С., 2018). Absorption in the  $3200\text{-}2800\text{ cm}^{-1}$  range is caused by the partial association of  $\text{-NH}_2$  groups,  $\text{-OH}$  groups of gallic acid anions as well as  $\text{NH}_4^+$  cations [Nakanishi K., 1962], component parts of Galmet preparation. A wide absorption band of medium intensity in the  $2800\text{-}1800\text{ cm}^{-1}$  range is attributed to the associated  $\nu(\text{OH})$  oscillations [Bellamy L. J., 1954; Tarasevich B.N., 2012]. The presence of  $\text{NH}_4^+$  cations is also confirmed by an intensive band observed at  $1404\text{ cm}^{-1}$  and attributed to  $\delta(\text{N-H})\text{ cm}^{-1}$  oscillations in ammonium salts [Nakanishi K., 1962; Tarasevich B.N., 2012]. The absorption bands at  $1544$  and  $1345\text{ cm}^{-1}$  are attributed to  $\nu_{as}(\text{C-O})$  and  $\nu_s(\text{C-O})$  oscillations of the  $\text{COO}^-$  anions, respectively [Bellamy L. J., 1954; Tarasevich B.N., 2012], and the  $875\text{ cm}^{-1}$  band indicates the character of the substitution in gallic acid - 3,4,5-substitute of the benzene ring (the presence of 2 hydrogen atoms isolated in this aromatic ring). The spectrum is recommended as a benchmark for quality control in the reproduction of the preparation.

As mentioned above, ROSs are formed in plant cells under normal conditions and participate in metabolic processes, growth and development [Mittler R., 2002]. However, there is an eruption of ROSs in plants under stress conditions, which, depending on their concentration, causes a different degree of oxidative destruction of cellular structures. Several chemical compounds, known as plant growth regulators or physiologically active substances (PGR, PHAS) modulate plant responses to biotic and abiotic stress at cellular, tissue and organ levels. Their exogenous application modifies the intensity of some key physiological mechanisms, including photosynthesis, nitrogen metabolism, proline metabolism, antioxidant defense systems, increasing plant tolerance to the action of stress-factor. The results of the biochemical analyzes conducted in this study (Table 1) showed that under favorable soil conditions, maize and soybean plants in the control group are characterized by an approximately equal content of Malonic di-aldehyde, which, on the other hand, indicates the existence of moderate oxidative stress, generated by high temperature and relatively low air humidity.

**Table 1:-** Malonic di-aldehyde content and activity of antioxidant protection enzymes in the leaves of maize and soybean plants, grown under optimal humidity conditions.

Series indices	Control	Thiourea		Galmet		Thiogalmet	
	M ± m	M ± m	Δ, %M*	M ± m	Δ, %M*	M ± m	Δ, %M*
<b><i>Zea mays</i> L. 'P458'</b>							
MDA, μM · g <sup>-1</sup> fr.m.	33,7±0,2	31,4±0,0	-6,6	26,8±0,1	-20,3	24,9±0,04	-26,0
SOD, conv. un. · g <sup>-1</sup> fr.m.	17,4±1,1	19,9±0,8	13,9	20,9±1,2	19,8	22,1±0,57	26,6
CAT, mM · g <sup>-1</sup> fr.m.	7,9±0,02	8,6±0,1	7,8	9,6±0,4	20,2	10,7±0,47	34,5
APX, mM · g <sup>-1</sup> fr.m.	4,2±0,1	5,5±0,5	30,9	5,4±0,2	28,6	5,6±0,26	32,3
GLPX, mM · g <sup>-1</sup> fr.m.	46,4±0,4	52,8±0,2	13,9	58,9±0,3	26,9	62,5±4,98	34,7
GLR, mM · g <sup>-1</sup> fr.m.	69,4±6,5	82,2±4,3	18,4	86,5±4,6	24,6	85,0±3,67	22,5
<b><i>Glycine max</i> (L.) Merr., 'Nadejda' variety</b>							
MDA, μM · g <sup>-1</sup> fr.m.	32,9±0,1	27,1±0,1	-17,6	26,2±0,1	-20,4	24,7±0,70	-24,9
SOD, conv. un. · g <sup>-1</sup> fr.m.	26,3±1,5	28,2±1,8	7,1	29,2±0,6	11,4	30,3±1,73	15,3
CAT, mM · g <sup>-1</sup> fr.m.	6,0±0,2	6,5±0,1	7,5	6,9±0,3	14,7	7,5±0,25	25,0
APX, mM · g <sup>-1</sup> fr.m.	5,3±0,2	6,0±0,1	14,4	6,5±0,8	23,2	6,5±0,27	23,7
GLPX, mM · g <sup>-1</sup> fr.m.	59,6±0,6	63,7±2,0	7,0	68,3±0,4	14,3	68,1±1,33	14,3
GLR, mM · g <sup>-1</sup> fr.m.	106,6±3,7	117,5±7,0	10,	121,6±4,5	14,1	126,6±3,2	18,7

\* - compared with the control plants; fr. m. – fresh mass

Under favorable humidity conditions, pre-treatment of seeds before sowing and plant leaf area during vegetative growth with Tu, Galmet and, in particular, with Thiogalmet conditioned a decrease in the MDA content for both crops: in pre-treated with Thiourea maize and soybean plants there was a decrease of 6.6% and 17.6% respectively. The use of Galmet solution for pre-treatment ensured a decrease in lipid peroxide oxidation processes and a reduction of Malonic di-aldehyde content by 20.32% compared to the values in the leaves of control group for both cultures. In the pre-treated maize and soybean plants with the new composition - Thiogalmet, the degree of decrease in the MDA content was 26.00% and 24.95%, respectively, compared to the leaf MDA content in plants of the control group. The use of Thiogalmet solution under normal humidity conditions reduced the MDA content in maize and soybean plants by 20.77% and 2.79% compared to the reduction degree conditioned by plant pre-treatment with Thiourea and by 7.12% and 5.8% compared to the series pre-treated with Galmet (Table. 1). Exogenous application of these preparations also induces a significant increase in the activity of the enzymatic system of antioxidant protection. The major effect of decreasing the MDA content in plants pre-treated with the indicated preparations is due to the more significant intensification of the activity of antioxidant protection enzymes. Thus, Thiogalmet composition ensured an increase of the SOD, CAT, APX, GLPX and GLR activity in maize plants by 9.45; 6.08; 8.23; 7.26 and 37.48% compared to the plants pre-treated with Thiourea. In soybean plants pre-treated with Thiogalmet, the degree of increase in the activity of these enzymes was respectively: 8.17; 31.63; 22.64; 16.06 and 15.10%.

It follows that Thiogalmet preparation possesses antioxidant properties and its use for plant pre-treatment under optimal humidity conditions ensures an improvement of the redox status by activating antioxidant enzymes and decreasing MDA content.

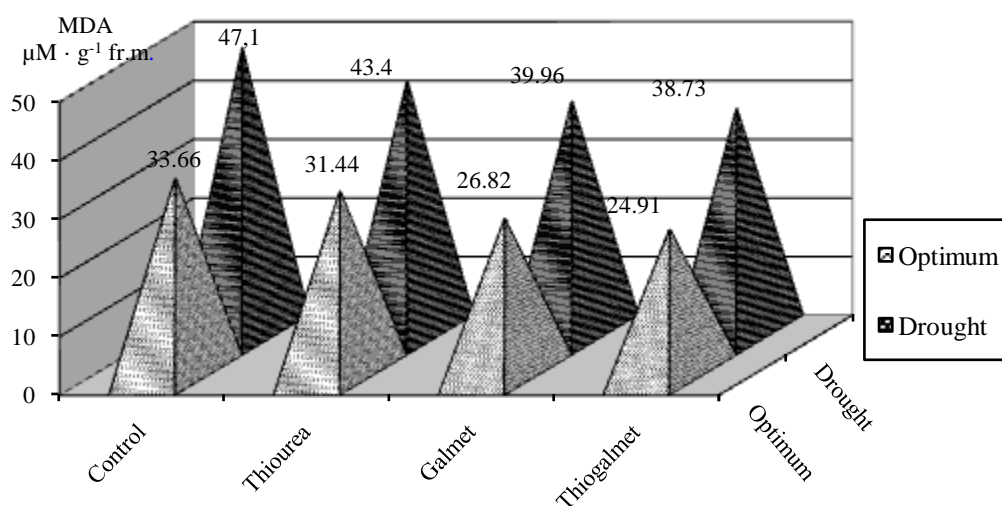
The beneficial effect of maize and soybean pre-treatment with the new composition was also recorded when determining the antioxidant properties of plants under conditions of oxidative stress caused by moderate moisture insufficiency (Table 2, Fig. 2 and 3).

**Table 2:-** Malonic di-aldehyde content and activity of antioxidant protection enzymes in the leaves of maize and soybean plants grown under moderate drought conditions.

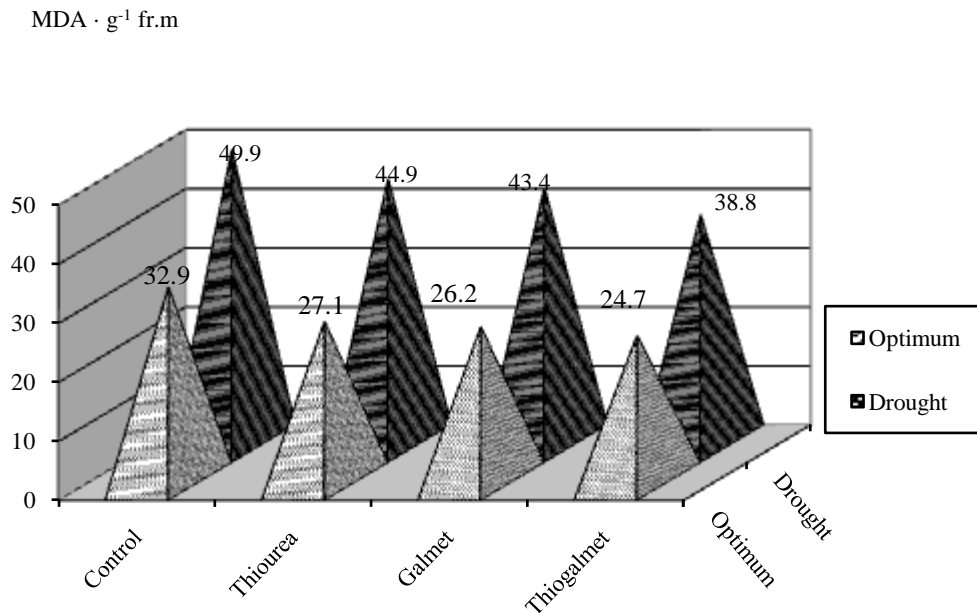
Series	Control	Thiourea		Galmet		Thiogalmet	
	M ± m	M ± m	Δ, %M*	M ± m	Δ, %M*	M ± m	Δ, %M*
<i>Zea mays</i> L. 'P458' cv.							
MDA, $\mu\text{M} \cdot \text{g}^{-1} \text{fr. m.}$	47,1±0,91	43,4±0,03	-7,85	39,9±0,2	-15,28	38,8±0,07	-17,62
SOD, conv. un. $\cdot \text{g}^{-1} \text{fr. m.}$	25,2±1,15	32,8±0,62	30,15	34,6±1,0	37,30	35,9±0,75	42,4
CAT, $\text{mM} \cdot \text{g}^{-1} \text{fr. m.}$	10,5±0,32	11,5±0,30	9,52	11,7±0,4	11,42	12,2±0,33	16,19
APX, $\text{mM} \cdot \text{g}^{-1} \text{fr. m.}$	7,0±0,28	8,5±0,78	21,43	9,0±0,4	28,57	9,2±0,30	31,42
GLPX, $\text{mM} \cdot \text{g}^{-1} \text{fr. m.}$	69,8±1,80	81,2±0,24	16,33	84,4±0,7	20,91	87,1±1,90	24,78
GLR, $\text{mM} \cdot \text{g}^{-1} \text{fr. m.}$	97,7±3,07	115,0±2,9	17,71	140,8±4,2	44,11	158,1±1,7	61,82
<i>Glycine max</i> (L.) Merr., 'Nadejda' variety							
MDA, $\mu\text{M} \cdot \text{g}^{-1} \text{fr. m.}$	49,9±0,40	44,9±0,04	-10,0	43,4±0,1	-13,08	38,8±0,1	-22,2
SOD, conv. un. $\cdot \text{g}^{-1} \text{fr. m.}$	39,8±1,04	47,7±2,42	19,66	51,6±1,5	29,64	51,6±0,6	29,64
CAT, $\text{mM} \cdot \text{g}^{-1} \text{fr. m.}$	7,7±0,02	9,8±0,42	27,90	11,5±0,3	49,35	12,9±0,6	67,53
APX, $\text{mM} \cdot \text{g}^{-1} \text{fr. m.}$	9,0±0,54	10,6±0,54	18,00	11,8±1,1	31,11	13,0±1,2	44,44
GLPX, $\text{mM} \cdot \text{g}^{-1} \text{fr. m.}$	86,7±1,70	102,1±4,1	17,83	114,7±1,6	32,29	118,5±2,2	36,67
GLR, $\text{mM} \cdot \text{g}^{-1} \text{fr. m.}$	147,9±1,9	156,2±2,7	5,59	169,8±2,4	14,81	179,8±8,0	21,57

\* - compared with the control plants; fr. m. – fresh mass

It has been shown that drought causes an increase in MDA content in the leaves of untreated maize and soybean plants by 39.84% and 51.67% of the values in control plants not exposed to drought. The MDA content of plants pre-treated with Thiourea, Galmet and Thiogalmet under conditions of moderate drought was maintained at a lower level by 7.85; 15.28 and 17.62% for maize and 10.0; 13.08 and 22.24% for soybean plants, compared to the values in untreated and exposed to drought plants. The MDA content in plants pre-treated with Thiourea and Thiogalmet due to insufficient moisture was higher than the MDA level in control plants under optimal humidity conditions by 28.78 and 14.92% for maize and by 36.47 and 17.93% in soybean plants, which represents a significantly lower degree of MDA content change compared to the untreated plants but exposed to the action of drought-induced oxidative stress.



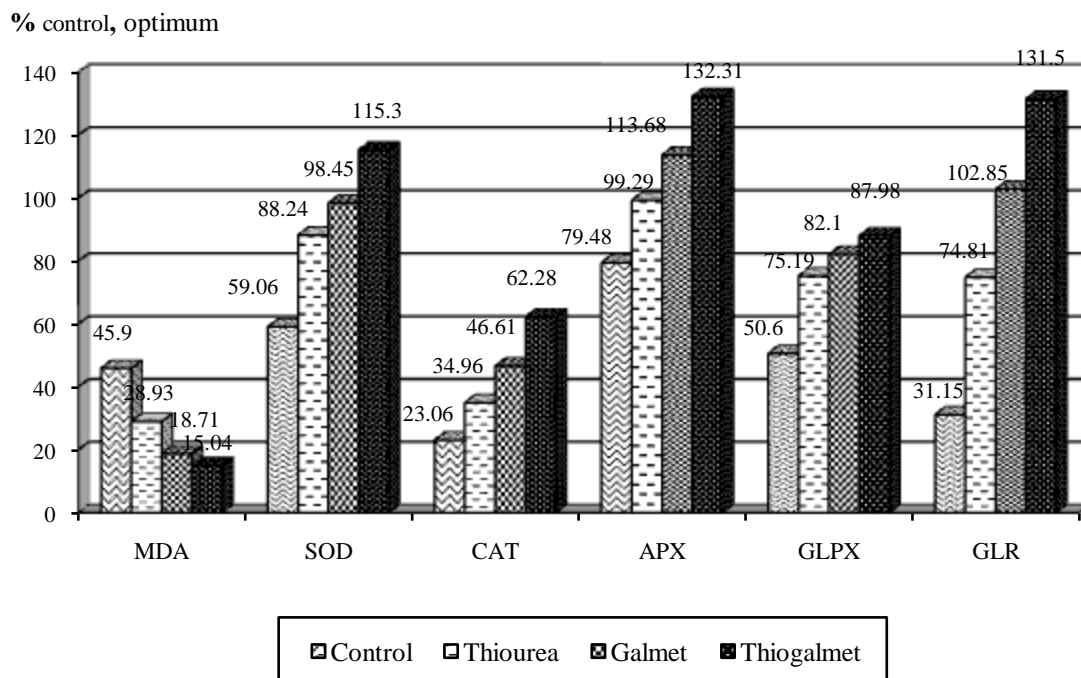
**Fig. 2:-** Malonic di-aldehyde content in the leaves of 'P 458' maize plants pre-treated with Thiogalmet under different humidity conditions.



**Fig. 3:-** Malonic di-aldehyde content in the leaves of soybean 'Nadejda' variety plants pre-treated with Thiogalmet depending on the humidity conditions.

Exogenous application of Thiogalmet conditioned a reduction of MDA content by 8.15 and 11.22% compared to maize and soybean plants pre-treated with Thiourea. As known from scientific sources, there are data that show that the activity of some physiologically active substances manifests itself differently depending on the conditions of the external environment. Thus, the use of Thiourea as an agent for seed and leaf pre-treatment or as an additional remedy is much more effective under stress than under normal conditions [Amin, A.A., A.A.Abd El-Kader, M.A.F. Shalaby, F.A. Gharib, and E. S. M. Rashad, 2013; Wahid A., et al., 2017]. Among the biochemical changes detected in different plant species, seed pre-treatment with Thiourea led to the induction of antioxidant protection and decrease of hydrogen peroxide and Malonic di-aldehyde synthesis [Perveen S., et al., 2016].

Maintaining the redox state in the pre-treated plants is conditioned by activation of plant enzymatic antioxidant protection system under both optimal conditions and oxidative stress (Fig.4; Table 3).



**Fig. 4:-** The degree of change in Malonic di-aldehyde content and activity of antioxidant protection enzymes (% of the index values in control plants under optimal conditions) in the leaves of maize 'P 458' plants under the influence of pre-treatment with Thiogalmet and exposure to drought.

Under optimal humidity conditions the activity of antioxidant enzymes increased in maize and soybean plants by 9.31 and 8.89% under the influence of Thiourea and by 24.1 and 14.4% respectively after pre-treatment with Galmet solution. Under these conditions, overall enzymatic activity in the maize and soybean plants increased respectively by 27.5 and 16.9% compared to the values in control series.

The degree of change in the activity of antioxidant enzymes conditioned by Thiogalmet pre-treatment compared to pre-treatment with Thiourea, known as a substance with antioxidant properties, for maize and soybeans under optimal humidity was 16.65 and 7.32%, and under conditions of drought - 20.60 and 6.04% respectively. The degree of change in the activity of antioxidant enzymes conditioned by Thiogalmet pre-treatment compared to pre-treatment with Thiourea under drought conditions was 20.60 and 6.04% in maize and soybean respectively and 7.1 and 4.9% compared to the Galmet pre-treated series. The new Thiogalmet preparation had significantly better antioxidant properties compared to Thiourea.

**Table 3:-** Degree of modification in MDA content and overall activity of antioxidant protection enzymes in the maize and soybean plants induced by pre-treatment under conditions of optimal humidity and moderate drought.

Series	Control		Thiourea		Galmet		Thiogalmet		
	M ± m	Δ, %M*	M ± m	Δ, %M*	M ± m	Δ, %M*	M ± m	Δ, %M*	
<b>Zea mays L. 'P458' cultivar</b>									
MDA, $\mu\text{M} \cdot \text{g}^{-1} \text{fr. m.}$	<i>optimum</i>	33,7±0,2		31,4±0,1	-6,59	26,8±0,1	-20,3	24,9±0,1	-25,99
	<i>drought</i>	47,1±0,9	39,8	43,4±0,2	-7,79	39,9±0,2	-18,63	39,8±0,1	-15,31
Overall enzymatic	<i>optimum</i>	145,4±8,4		158,9±6,8	9,31	180,5±6,4	24,1	185,4±9,9	27,51

activity	<i>drought</i>	210,2±6,6	44,5	249,0±6,7	18,44	280,5±6,7	33,4	300,3±4,9	42,85
<b><i>Glycine max</i> (L.) Merr., 'Nadejda' variety</b>									
MDA, $\mu\text{M} \cdot \text{g}^{-1} \text{fr. m.}$	<i>optimum</i>	32,9±0,1		25,4±0,1	-22,78	26,2±0,1	-20,4	24,7±0,7	-24,95
	<i>drought</i>	49,9±0,4	51,8	42,7±0,1	-14,40	43,4±0,1	-13,1	37,9±0,1	-24,00
Overall enzymatic activity	<i>optimum</i>	203,7±6,5		221,9±6,5	8,89	232,5±6,6	14,14	238,1±6,8	16,87
	<i>drought</i>	291,2±5,3	42,95	326,4±6,9	12,13	339,4±6,9	16,55	356,2±9,5	18,89

$\Delta$ , % M \*\* - compared to control plants under corresponding conditions

Therefore, the obtained results show that plant pre-treatment with Tu, Galmet and, in particular, with Thiogalmet increase the activity of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GLR) and glutathione peroxidase (GLPX). High activity of antioxidant enzymes is associated with decreased malonic di-aldehyde (DAM) content. The beneficial physiological effect of Thiogalmet is also confirmed by the higher levels of the assimilation processes and plant growth.

The use of Thiogalmet preparation for seed material and leaf pre-treatment is more effective for improving the biological performance of *Z. mays* plants by increasing the activity of antioxidant enzymes, photosynthesis and plant productivity.

### Conclusions:-

1. Thiogalmet, which containing Thiourea and Galmet - a preparation that consists of a mixture of potassium, ammonium, magnesium gallates, potassium molybdate and ammonium paramolybdate, is a complex physiologically active compound that possesses antioxidant properties.
2. The plants treated with Thiogalmet possess a significantly higher capacity of antioxidant protection compared to the plants treated with Thiourea and Galmet, and, in particular, with those from the control group.
3. Thiogalmet can be used to reduce the negative impact of oxidative stress caused by reactive oxygen species (ROS), for antioxidant protection and reduce the oxidative destruction of plant cellular components under drought conditions.
4. The IR spectrum of the preparation Thiogalmet in the region 400-4000  $\text{cm}^{-1}$  demonstrates that when homogenizing the components of the preparation (Thiourea and Galmet), the spectral changes are non-essential and refer to the association of groups of atoms ( $\text{NH}_2^-$ ,  $\text{OH}$ ,  $\text{NH}_4^+$  and other) by forming hydrogen bonds. The spectrum is recommended as a benchmark for quality control in the reproduction of the preparation.

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### References:-

1. Amin, A.A., A.A. Abd El-Kader, M.A.F. Shalaby, F.A. Gharib, and E.S.M. Rashad. Physiological Effects of Salicylic Acid and Thiourea on Growth and Productivity of Maize Plants in Sandy Soil. //In: Soil Science and Plant Analyses. 2013. Pp. 1141-1155
2. Asada K. Production and scavenging of reactive oxygen species in chloroplasts and their functions. Published in: Plant Physiology, 141. No. 2. Pp. 391-396
3. Bartolia C. G., Casalongué C. A., Simontacchia M., Marquez-Garcia B., Foyer C.H. Interactions between hormone and redox signaling pathways in the control of growth and cross tolerance to stress. // Environmental and Experimental Botany. 2013. V. 94. P. 73-88. /Cross-stress tolerance and stress "memory" in plants. Edited by Dr. Sergi Munne-Bosch and Dr. Leonor Alegre.
4. Bates L., Waldren R.P., Teare I.D. Rapid determination of free proline for water-stress studies. Plant and Soil, 1973, 39. Pp. 205-207.

5. Beck E.H., Fettig S., Knake K., et al. Specific and unspecific responses of plants to old and drought stress. In: Journal of Biosciences. 2007.V. 32. Pp. 501-510
6. Becana M., Aparicio-Tejo P.M., Irigoyen J.J., Sanchez-Diaz M. Some enzymes of hydrogen peroxide metabolism in leaves and root nodules of *Medicago sativa* // Plant Physiol. 1986. V. 82. Pp. 1169-1171.
7. Bellamy L. J. Infrared spectra of complex. New York, USA. Wiley, 1954
8. Bharti Badhani, Neha Sharma and Rita Kakkar. Gallic acid: A Versatile Antioxidant with Promising Therapeutic and Industrial Applications. RSC Adv., 2015. 5. Pp. 27540-27557
9. Bhattacharjee S. Reactive oxygen species and oxidative burst: role in stress, senescence and signal transduction in plants. // Current Science. 2005, 89(7). Pp. 1113-1121.
10. Bhattacharjee Soumen. The Language of Reactive Oxygen Species Signaling in Plants. // Journal of Botany. V. 2012. 22 p.
11. Chance B. și Machly A. Assay of catalases and peroxidases. Methods in Enzymology, S.P. Colowick and N.O. Kaplan (ed). N.Y.: Acad. Press, 2. 1955. Pp. 764-775.
12. Coropceanu, Ciloci, Ștefăriță, Bulhac, Study of useful properties of some coordination compounds containing oxime ligands. Published in: Academica Greifswald, Germany. 2020. 266 p.
13. Foyer C.H., Shigeoka S. Understanding Oxidative Stress and Antioxidant Functions to Enhance Photosynthesis. // Plant Physiology. 2011. 155. Pp. 93-100.
14. IPCC Special Report on Managing the Risks of Extreme Events and Disasters to Advance Climate Change Adaptation. Facts Sheet. [http://www.ipcc.ch/news\\_and\\_events/docs/srex/SREX\\_fact\\_sheet.pdf](http://www.ipcc.ch/news_and_events/docs/srex/SREX_fact_sheet.pdf)
15. Jentsch A., Kreyling J., Elmer M., Gellesch E., et al., Climate extremes initiate ecosystem-regulating functions while maintaining productivity Published in: Journal of Ecology. 2011. V. 99. Pp. 689-702.
16. Keshavkant S., Naithani S.C., Chilling induced superoxide production, lipid peroxidation and leakage loss in *Shorea robusta* seedlings. Published in: Indian J. Plant Physiol. V. 2010. 15. Issue 2. Pp. 192-197.
17. Krasensky H., Jonak C., Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. Published in: Journal of Experimental Botany, 2012. V. 63. No. 4. Pp. 1593-1608
18. Merewitz E.B., Gianfagna T. și Huang B. Protein Accumulation in Leaves and Roots Associated with Improved Drought Tolerance in Creeping Bentgrass Expressing in an *ipt* Gene for Cytokinin Synthesis // J. Exp. Bot. 2011. 62. Pp. 5311-5333
19. Mittler R., Blumwald E. Genetic engineering for modern agriculture: challenges and perspectives. // Annual Review of Plant Biology. 2010. V. 61. Pp. 443-462.
20. Munné-Bosch S., Queval G., Foyer C.H. The impact of global change factors on redox signaling underpinning stress tolerance. Plant Physiol. 2013. 161. Pp. 5-19
21. Nacano Y., Asada K. Hydrogen Peroxide Is Scavenged by Ascorbate Specific Peroxidase in Spinach Chloroplasts. Published in: Plant Cell Physiol. 1981. 22. Pp. 867-880.
22. Nakanishi K. Absorption spectroscopy. Tokyo, Japan. Holden Day 1962.
23. Pandey, M., Srivastava, A.K., D'Souza, S.F. & Penna, S. Thiourea, a ROS scavenger, regulates source-to-sink relationship to enhance crop yield and oil content in *Brassica juncea* (L.). PLoS One, 2013. 8(9): e73921.
24. Parisa Sharifi, Reza Amirnia, Eslam Majidi, Hashem Hadi, Foad Moradi, Mozafar Roustaei, Jafar Jafarzadeh. Comparative analysis of phytohormones and oxidative damage in flag leaves of six contrasting wheat genotypes in response to drought stress. Advances in Environmental Biology. 2012. 6(4). Pp. 1540- 1551
25. Perveen A., Wahid A., Mahmood S., Hussain I., Rasheed R. Possible mechanism of medium-supplemented thiourea in improving growth, gas exchange, and photosynthetic pigments in cadmium-stressed maize (*Zea mays*). Brazilian J. Bot. 2015. 38. Pp. 71-79. Doi: 10.1007/s40415-014-0124-8.
26. Perveen, S., Farooq, R., Shahbaz, M. Thiourea-induced metabolic changes in two mung beans [*Vigna radiata* (L.) Wilczek] (Fabaceae) varieties under salt stress. Brazilian J. Bot. 2016. 39. Pp. 41-54. Doi: 10.1007/s40415-015-0209-z
27. Schadle M., Bassham J.A., Chloroplast glutathione reductase. Published in: Plant Physiol. 1977.V. 59. P. 1011-1012.
28. Singh Manish Pal\*, Gupta Avneet, Sisodia S Siddhraj. Gallic Acid: Pharmacological Promising Lead Molecule. // International Journal of Pharmacognosy and Phytochemical Research 2018; 10(4); Pp. 132-138 doi: 10.25258/phyto.10.4.2 ISSN: 0975-4873
29. Ștefăriță A. Semnificația apei în coordonarea și integrarea funcțiilor plantei în condiții de secetă. // Buletinul Academiei de Științe a Moldovei. Științele vieții. 2012, 1(316). Pp. 38-53. (In Romanian)
30. Ștefăriță A., Botnari V., Brînză L., Bulhac I., Coropceanu E., Bouroș P., Chilinciu A. Possibilities of increasing the antioxidant properties of garlic plants (*Allium sativum* L.). Published in: Acta Chemica. 2017. Iași. nr. 25, 2. Pp. 208-231

31. Ștefîrță A., Brînză L., Toma S., Bulhac I., și al. Opțiuni fiziologice de fortificare a plantelor în condiții de insuficiență de umiditate. //În: Diminuarea impactului factorilor pedoclimatici extremali asupra plantelor de cultură. Chișinău. 2008. Pp. 166 -203 (In Romanian)
32. Ștefîrță A., Bulhac I., Coropceanu E., Voloșciuc L., Brînză L. Effect of Cytokinin-Type Compounds on The Self-Regulation of Plant Water Status Under Conditions of Adverse Humidity Variation and Repeated Water Stress. In: SSRG International Journal of Agriculture and Environmental Science. Volume 8 Issue 3. Pp. 1-7. May-June 2021. ISSN: 2394 –2568 /doi:10.14445/23942568/IJAES-V8I3P101 IF 1,19
33. Ștefîrță A., Bulhac I., Coropceanu E., Brînză L. The action of some cytokinin-type compounds on the antioxidant protection capacity of plants in conditions of unfavorable variation of humidity and repeated water stress. In: International Journal of Advanced Researches. 2021. Volume 9. ISSUE 7. Pp. 642-653. Article DOI: 10.21474/IJAR 01/ 13166/ DOI URL: [http:// dx/Doi/org/10.21474/IJAR01/13166](http://dx/Doi/org/10.21474/IJAR01/13166). IF- 7, 337. ISSN 2320-5407.
34. Ștefîrță A.; Bulhac I., Coropceanu E., Brînză L. Polyel – compound with antioxidant properties. In: Journal of Applied Life Sciences and Environment (ALSE). 2021. Volume LIV. Issue 2 (186). P. 146-155. <https://doi.org/10.46909/journalalse-2021-014>
35. Ștefîrță A., Leahu I., Toma S. Răspunsul specific și nespecific al plantelor la acțiunea stresului hidric și termic: relațiile interactive dintre status-ul apei și protecția antioxidantă. In: Buletinul Academiei de Științe a Moldovei. Științele vieții. 2015. V.1, p.29-46. (In Romanian)
36. Wahid, A., S.M.A. Basra and M. Farooq, Thiourea: a molecule with immense biological significance for plants. //Int. J. Agric. Biol., 2017. 19. P. 911-920 <http://www.fspublishers.org>
37. Walter Julia , Anke Jentsch, Carl Beierkuhnlein, Juergen Kreyling. Cross-stress tolerance and stress "memory" in plants //Environmental and Experimental Botany. 2013. V. 94. P. 3-8.
38. Zhongbing Lu, Guangjun Ni, Peter S. Belton, Huiru Tang, Bao Zhao. Structure–activity relationship analysis of antioxidant ability and neuroprotective effect of gallic acid derivatives // Neurochemistry International 2006. 48. Pp. 63-274
39. Isacov H., Ascarov I., Usmanov C. IR Spectroscopic Studies of Thiourea-Formaldehyde Oligomers Compounds // Universum: Engineering sciences: electron. scientific journal. 2018. № 12(57). URL: <http://7universum.com/ru/tech/archive/item/6746>. (In Russian)
40. Laman H., Mendeli D., Calățcaia J. Adaptogenic effect of phenyl urea derivatives on the formation of productivity of spring barley plants. In: Plant and stress. Moscow, 2010. C. 215 (In Russian)
41. Tarasevich B.N. IR spectra of the main classes of organic compounds. Reference materials. Moscow. Russia. Izdatelstvo inostranoi literature 2012. (In Russian).