SECTION 1. AGRONOMY

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1.1 Efficient growth-promoting agents: thiosulfonate and biocouple combinations

Without the use of modern agricultural chemicals, it is impossible to obtain high yields of many important crops. Along with the use of mineral and organic fertilizers, herbicides and pesticides, plant protection products, the use of plant growth regulators is also of great importance [1].

Modern plant growth regulators are indispensable for increasing seed germination and germination energy. They can boost plant immunity, resistance to unfavorable growing conditions and stressful situations, accelerate flowering and fruiting, increase yields, and ensure the ecological purity of the harvest [1].

Recently, a significant number of drugs have appeared that are called immunomodulators or phytohormones, so one might think that these drugs save from all kinds of diseases and pests. That is why it is important to clearly understand what these drugs are, how effective they are, how they differ, and how they are used. All these drugs are plant growth regulators. Suppressing pests and inhibiting the action of plant pathogens (fungi, bacteria, and viruses) is not the main task of these drugs. First and foremost, they are powerful biostimulants [1].

It should be noted that growth regulators only have a positive effect if the general principles of growing and caring for specific plants are not violated. Thus, if the technology of crop cultivation is violated, weeds, pests, and diseases are not controlled, and plants lack macro- and microelements of mineral nutrition, then no preparations, including growth regulators, will help increase the yield [1].

Among the many definitions of growth regulators, the one given by L. J. Nickell is particularly informative. Plant growth regulators are natural or synthetic chemicals used to treat plants in order to alter their life processes or structure to improve their quality, increase yield, or facilitate harvesting. Phytohormones are chemicals produced in plants that coordinate their growth and development. Thus, the term "growth regulator" includes natural phytohormones and synthetic compounds [2].

Plant hormones were discovered relatively recently. In the 1920s and 1930s, interest in research in this area grew when the hormone indole-3-acetic acid (IAA) was isolated. Research was conducted in the Netherlands by a group of scientists led by F. Vent, F. Kegle, and A. Hagen-Smith. At about the same time, Japanese researcher E. Kurosawa studied substances that cause hypertrophic growth in rice, now known as gibberellin phytohormones. Soviet scientists also made a significant contribution to the development of the theory of hormonal regulation: biologist N.G. Kholodny, botanist N.A. Maksimov, physiologist M.Kh. Chaylakhian, and others [2].

According to modern theory, phytohormones regulate numerous processes of plant life at all stages of life – from embryo development to the complete end of the life cycle. The use of regulators allows changing the processes of plant growth and development, controlling the processes of flowering, transition to dormancy and emergence from it, and organ formation [2].

Growth regulators act similarly to mammalian hormones, exhibiting high biological activity. This allows them to be used in low doses – at the level of grams or milligrams of active ingredient per hectare [2].

Plant hormones are classified according to their chemical nature or physiological effect. Growth regulators can be divided into stimulating substances – activators – and compounds that inhibit physiological and biochemical processes – inhibitors. Growth activators include auxins, gibberellins, and cytokinins, while abscisic acid and ethylene inhibit plant growth and development processes [2].

The mechanism of action of phytohormones is largely similar to that of animal hormones, although it is less well understood. Each hormone has its own specific receptor located in the membranes of target cells. The mechanism of action of some hormones is based on their ability to penetrate the cell and form a complex with nuclear receptors there. Other hormones are capable of altering the activity of metabolic stages or the cytoplasmic concentration of a particular ion, resulting in a cellular response. The signaling system of plant cells receives, amplifies, and transmits the primary intracellular signal to the genome. During signal transmission within the cell, secondary small molecules, also called messengers, are involved in the process. As a result of their participation, the signal spreads faster throughout the cell. Messengers can control many processes in the cell. Among the many messengers, calcium cation can be singled out, as it is considered universal [2].

Today, plant growth regulators have found practical application in the following main areas [1]:

• revitalization of weakened and rejuvenation of old plants by stimulating shoot formation and root system development;

• restoring damaged plants after stress (planting, transplanting, storage, long-term transportation, suboptimal lighting and temperature, pesticide treatment, soil salinity, etc.);

• stimulation of early and abundant flowering, intense coloring of leaves and flowers by enhancing the synthesis of chlorophyll and other pigments;

• induction of increased resistance to phytopathogens (especially root rot), pests, and unfavorable growing conditions;

• stimulation of active growth of vegetative mass;

• activation of the enzymatic and hormonal systems of plants, etc.

When using growth regulators, it is necessary to take into account that each of them is designed to stimulate the growth, development, and productivity of certain crops at appropriate doses, times, and methods of application [1, 2].

Natural growth regulators and their application. Natural phytohormones vary in chemical structure and effects. The first natural hormone was discovered by Charles Darwin while studying phototropism. Studying the effect of light on plant growth in his book “The Power of Movement in Plants,” he noticed that when a seedling is freely placed on a windowsill, a signal is transmitted from the upper part of the plant to the lower part, causing the latter to turn toward the light. He concluded that only the top of the seedling perceives the direction of light and transmits information to neighboring parts of the plant. Further research established the chemical nature and physiological effects of the substance that actually has this effect on the plant. The discovered hormone was named “auxin” and was identified as indoleacetic acid (IAA). This phytohormone stimulates cell growth in the elongation zone, leading to an increase in shoots. At the molecular level, it affects the processes of cell differentiation, respiration, photosynthesis, and the activity of the protein synthesis apparatus [3].

Auxins are constantly produced in various parts of young plants. They are found in large quantities in seeds and fruits. The amount of auxins changes throughout the life of plants. Their content peaks when the plant enters the budding or flowering phase, and conversely, their amount decreases when growth stops [3].

The largest group of phytohormones is gibberellins. In Japan, there is a widespread rice disease that locals call “bakanoe” – “mad rice” or “bad sprouts.” Seedlings of diseased plants outgrow healthy rice, but the ears grow deformed and without grain. In 1926, Japanese botanist E. Kurosawa isolated and described the causative agent of the disease, the fungus Gibberella fujikuroi (now transferred to the genus Fusarium). It soon became clear that many of the symptoms of “mad rice” could be induced by the culture fluid in which the fungus grew. This means that the fungus secretes a water-soluble substance that enhances rice growth. The substance was named gibberellin after the genus name of the fungus [1, 3].

Throughout the plant's life cycle, the set of gibberellins can change, and they all have different meanings for its development. Their action is similar to that of auxins, i.e., they cause stem elongation due to cell stretching. However, gibberellins are capable of enhancing not only stem growth but also changing the shape and number of leaves. The use of gibberellins in plant treatment helps them accumulate green mass and dry matter. A significant amount of gibberellins is produced in leaves and seeds [3].

The first cytokinin was discovered by Skog and Miller in 1955. In terms of its chemical structure, the isolated substance was similar to adenine and was named “kinetin.” Subsequently, its chemical analogues were grouped under the general name cytokinins [3]. As a result of treating plants with cytokinins, the dormant phase is interrupted and the plants begin to sprout with greater vigor. Numerous experiments have demonstrated the ability of cytokinins to activate cell division and increase the number of mitoses [2].

Treatment with cytokinins leads to an increase in germination energy, seed germination, dry weight accumulation by seedlings, and termination of the dormancy phase of dormant buds in woody plants. A special effect is the stimulation of lateral shoot growth, the influence on the formation and development of generative organs, which causes plants to flower in unfavorable conditions. Cytokinins have a valuable property—they slow down the aging process in plants. They are able to attract nutrients to the spent organ. A high content of cytokinins can be observed in unripe fruits and developing seeds, as they are strongly associated with embryo development. They have also been found in shoots, and researchers often believed that they were also present in the roots [3].

Abscisic acid (ABA), a natural phytoregulator of terpenoid nature, has a wide range of effects. Its use can cause both inhibition and enhancement of processes in plants. Therefore, ABA can be considered an antagonist of substances that stimulate life processes in plants [3].

Abscisic acid slows down all growth processes, which is associated with the inhibition of nucleic acid production. ABA affects the photosynthetic function of plants, reducing chlorophyll content and photosynthetic activity. Abscisic acid is produced in cells in response to drying and cooling. In these conditions, water deficiency is observed, which is why this hormone is called the stress hormone. An important property of ABA is to ensure the winter dormancy of buds and seeds [3].

Ethylene is a regulator that, like ABA, inhibits plant growth and development processes and is capable of activating the aging process of cells, tissues, and organs. Thus, it accelerates the processes of wilting, flower fall, and the formation of the color characteristic of ripe fruits. In plants, ethylene can be produced in response to extreme temperatures, poor water supply, and mechanical trauma. Due to this property, the hormone has become very important in plant damage, which is why it is called the mechanical stress hormone [3].

In addition to the phytohormones listed above, there are endogenous substances known to have similar effects to phytohormones. Among them are polysaccharide compounds with growth-regulating properties. These substances participate in glycobiological interactions and can influence intracellular signaling processes. These include carbohydrate-binding proteins such as lectin and antibodies against carbohydrate determinants, membrane-bound glycoconjugates such as glycoproteins, glycolipids, oligo- and polysaccharides [3].

Synthetic growth regulators and their application. Synthetic growth regulators began to appear after the synthesis of auxin (indoleacetic acid) by Dutch plant physiologist F. Kegle (1931-1935). Subsequently, similar compounds with high biological activity were synthesized. The most promising growth stimulants were indolebutyric acid, naphthylacetic acid (NPA), and 2,4-dichlorophenylacetic acid (2,4-D). In 1955, kinetin (cytokinin) was synthesized [4].

Synthetic regulators also include plant growth inhibitors: retardants—preparations that reduce the length and increase the thickness of stems, and morphactins—compounds that cause abnormalities at the growth point and the appearance of distorted organs in plants. They also include substances that specifically inhibit the movement of IOC and its derivatives throughout the plant. Substances with a sharply inhibitory effect include herbicides that destroy weeds [4].

Unlike natural inhibitors, synthetic inhibitors are capable of suppressing growth processes more sharply. They are not subject to inactivation by plant tissues for a long period of time. Their action is often associated not only with growth, but also with the disruption of morphogenetic processes [5].

The use of plant growth regulators in practice allows for changes in metabolism identical to those that occur under the influence of certain external conditions (daylight hours, temperature, etc.), for example, to accelerate the formation of generative organs, enhance or inhibit growth, etc. To enhance the growth and organogenesis of cultivated plants, stimulants such as auxins and gibberellins are used, and to inhibit growth, synthetic growth inhibitors are used, including defoliants, which cause leaf fall, and desiccants, which dry out organs or entire plants [4].

Synthetic auxin-type stimulants (β-indoleacetic acid, or heteroauxin, β-indolebutyric acid, α-naphthylacetic acid) are used to enhance root formation in cuttings of woody and herbaceous plants, improve tissue fusion during grafting, and prevent fruit drop in fruit trees and berry bushes. These substances are used in various concentrations (from 20 to 1000 mg/l) depending on the method of application to the plant [4].

Gibberellins are used to enhance the growth of seedless grape varieties, bring potato tubers out of dormancy, enhance the growth of hemp and flax stems, and accelerate tomato fruiting [4].

Synthetic growth inhibitors are used to delay the sprouting of potato tubers during storage and to inhibit the growth of cereal stems to increase resistance to lodging. The mechanism of the inhibitory effect of synthetic inhibitors on plants has not been sufficiently studied. It has been established that most of them inhibit growth by separating the processes of phosphorylation and respiration and suppressing the synthesis of nucleic acids [4].

Among the growth regulators used in agricultural production, a major role belongs to preparations with a complex effect, which in some cases exceed the effectiveness of natural hormones or their synthetic analogues, which determines the timing of treatment (phases of crop development) and application rates. Thus, preparations based on triterpenic acids, used for seed treatment, exhibit the properties of auxins to a greater extent, and in the flowering phase (the beginning of fruit formation) – gibberellins. Ambiole works in a similar way – when treating seeds, it exhibits the properties of cytokinins, and subsequently – auxins. In some cases, the different properties of the preparation work simultaneously, ensuring its maximum effect [1].

Compounds of the 1,3,5-triazine series are characterized by a wide range of useful properties for practical use. They have found application in medicine, pharmacology, microbiology, agriculture, and the photo and textile industries. Today, 1,3,5-triazine derivatives are most widely used as herbicides (atrazine, simazine, propazine, promethrin) and in the production of resins (allyltriazine, vinyltriazine, and melamine-formaldehyde resins) [6,7]. 1,3,5-triazines are also known as fungicides, medicines, bleaches, disinfectants, stabilizers for lubricants, oils, and polymer materials. They are used in the production of dyes, explosives, and as condensation reagents in peptide synthesis [7]. The 1,3,5-triazine cycle is part of the structure of compounds that have anticancer, antiviral, antimicrobial, cardioprotective, anti-inflammatory, antituberculosis, antimalarial, antioxidant, and other types of activity [8]. Among the herbicides derived from triazine, there are broad-spectrum preparations capable of destroying many types of weeds, as well as preparations with narrow selectivity. They are characterized by both systemic and contact action [9].

All triazine herbicides are low-toxic to warm-blooded animals and humans. They penetrate plants better through the root system and reach the leaves with transpiration flow. Therefore, most of them are soil-acting herbicides. Only Semeron and certain formulations of Atrazine (Mayazin, Zeapos) are used against vegetative weeds [10].

The herbicidal activity of sim-triazines is directed against annual monocotyledonous and dicotyledonous weed species during their germination, but they are not sufficiently effective against rooted and perennial species. Triazines, for example, actively suppress Canadian thistle, burdock, white pigweed, wild radish, field mustard, chickweed, black nightshade, ragweed, broomweed, romen, chicken millet, galinsoga, and oatgrass [10, 11].

In the production of growth-stimulating preparations, an important task is to increase the solubility of active compounds in water. The solution to this problem may be the use of surfactants (SAs), which will act as emulsifiers, solubilizers, and stabilizers of the preparation.

Recently, surfactants of biogenic origin, or biosurfactants, have attracted particular interest. They are as effective as synthetic surfactants and, at the same time, are environmentally safe. Due to their physicochemical properties and biological activity, biogenic surfactants can enhance the effectiveness of growth-stimulating compounds [12].

Synthesized thiosulfonates derived from sim-triazine are promising for the creation of new plant growth stimulants, but their use is complicated by low solubility in water.

This problem can be solved by using surfactants that increase the solubility of thiosulfonates. This will also reduce the minimum amount of active ingredient required for effective application, which is important from an environmental and economic point of view.

To obtain a composition with growth-stimulating activity, we chose the rhamnolipid biocomplex Bacillus spp. as a surfactant. This type of biogenic surfactant has a number of important properties, among which the following are worth highlighting: surface and emulsifying activity, wetting of surfaces, and influence on the permeability of cell membranes. These properties allow biogenic surfactants to be used to create complex preparations with various biologically active substances, including poorly soluble thiosulfate esters [13-15].

Biogenic surfactants have certain advantages over synthetic ones—they are low-toxic and biodegradable, which determines their use in environmentally safe technologies. In our case, another important aspect is that the selected rhamnolipid surfactants are capable of affecting the permeability of plant cell membranes, which contributes to improved absorption of water and nutrients from the growing medium [16].

The composite preparations were developed based on synthesized 2,4-dichloro-6-(4′-aminobenzenesulfonylthiomethyl)-sym-triazine (MTS derivative of sym-triazine) or 2,4-dichloro-6-(4′-aminobenzenesulfonylthioethyl)-sym-triazine (ETS derivative of sim-triazine) and laboratory samples of rhamnolipid biocomplex concentrate (RBC). During the research, a number of water-alcohol complex preparations with different ratios of active compounds were obtained.

The main task in the development of complex preparations was to establish the optimal ratio of thiosulfoesters and bio-PAPs to create stable forms of preparations while maintaining their maximum effectiveness as plant growth regulators.

To study the obtained complex preparations, the UV spectra of freshly prepared solutions of thiosulfate esters, RBC, and their compositions were analyzed. The results of the research are shown in Figs. 1-3.

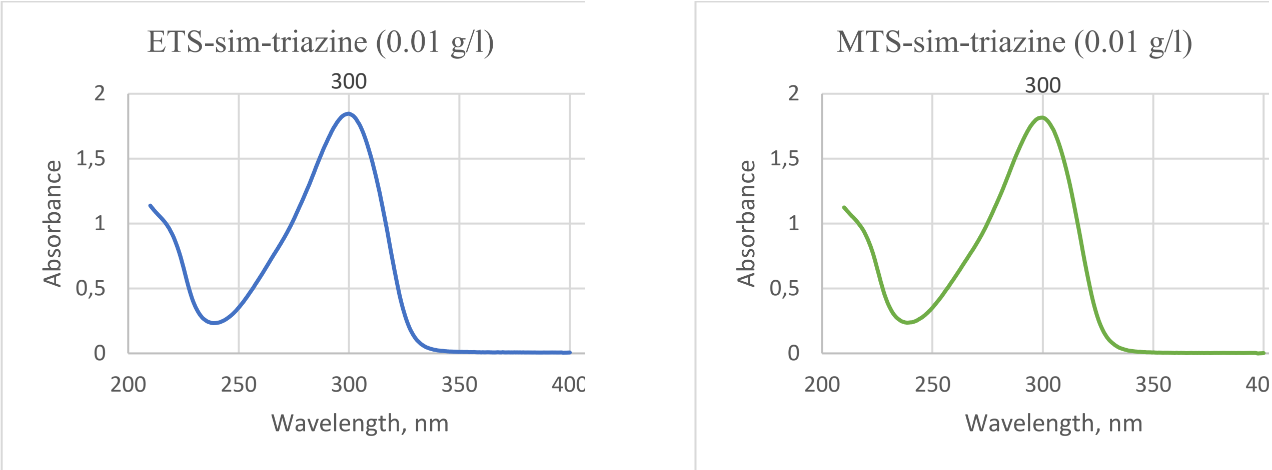

Figure 1. UV spectra of freshly prepared solutions of ETS-sym-triazine and MTS-sym-triazine with concentrations of 0.01 g/L.

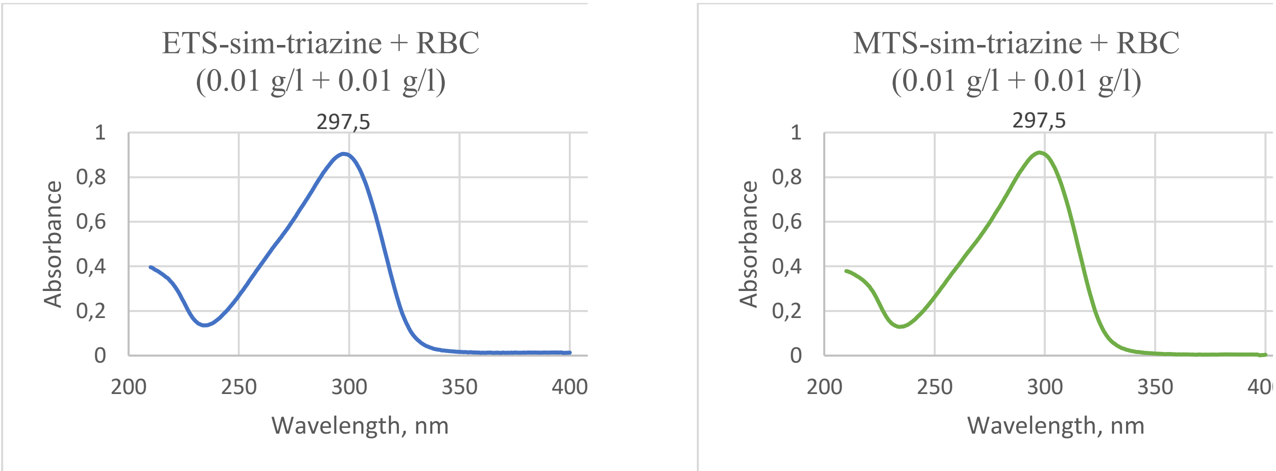

Figure 2. UV spectra of freshly prepared complex preparations of ETS-sym-triazine + RBC and MTS-sym-triazine + RBC with concentrations of 0.01 g/l + 0.01 g/l.

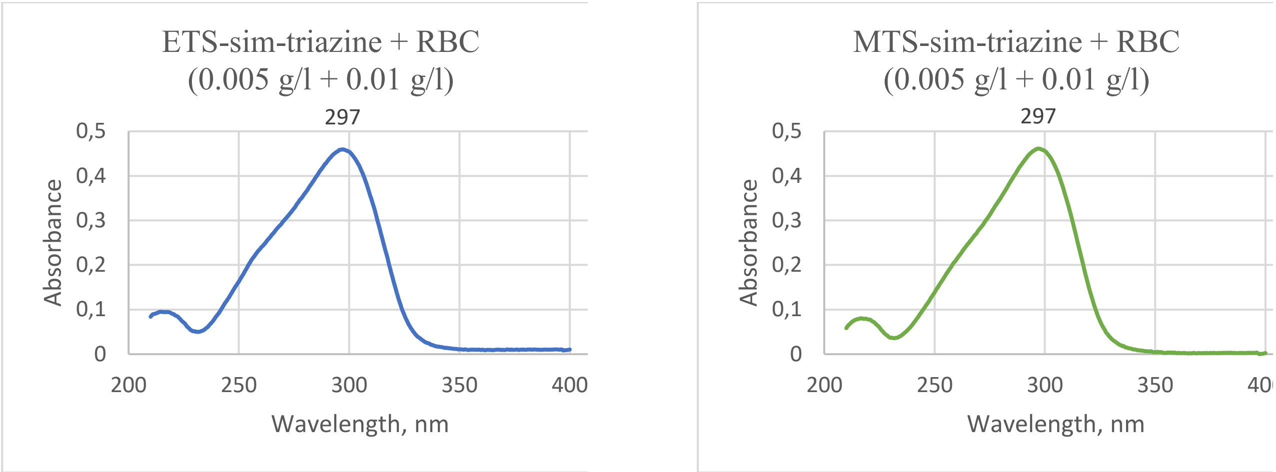

Figure 3. UV spectra of freshly prepared complex preparations of ETS-sym-triazine + RBC and MTS-sym-triazine + RBC with concentrations of 0.005 g/l + 0.01 g/l.

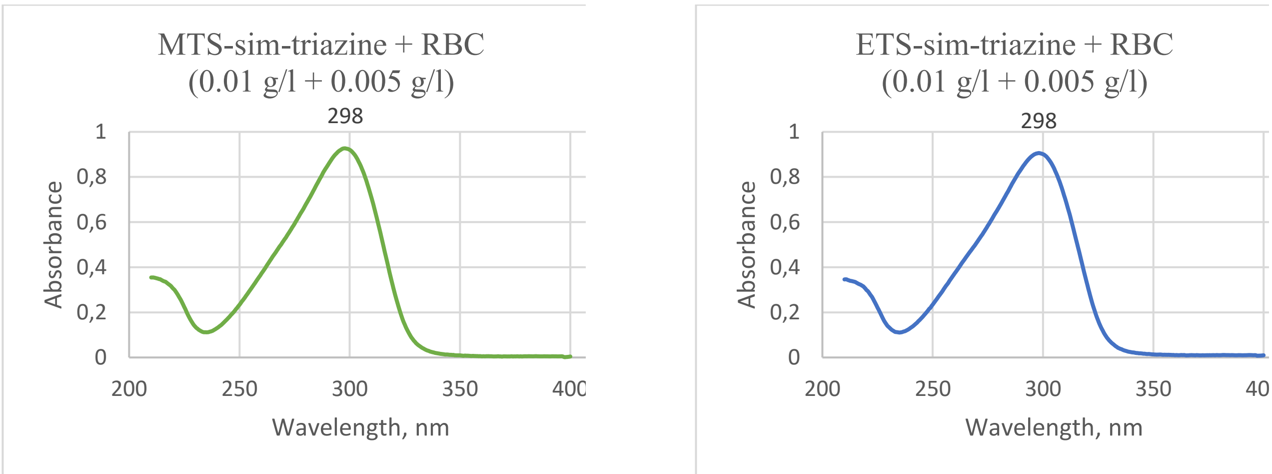

Figure 4. UV spectra of freshly prepared complex preparations of ETS-sym-triazine + RBC and MTS-sym-triazine + RBC with concentrations of 0.01 g/l + 0.005 g/l.

From the spectra obtained, it can be concluded that for individual substances, ETS-sym-triazine and MTS-sym-triazine, the absorption spectra in 96% ethanol are characterized by a single absorption band with a maximum at 300 nm.

The individual spectrum of RBC in both water and 96% ethanol does not contain peaks in the studied UV region.

No new peaks are observed in the spectra of the complex preparations ETS-sym-triazine + RBC and ETS-sym-triazine + RBC of all combinations. However, the absorption maximum is slightly shifted compared to the absorption bands characteristic of individual compounds and is λ = 297-298 nm. At the same time, the maxima of the spectra of the complex preparations ETS-sym-triazine + RBC coincide with the maxima of the spectra of the complex preparations MTS-sym-triazine + RBC with identical concentrations of active substances.

The data obtained indicate that in freshly prepared complex preparations, there is an interaction between thiosulfonates and RBCs with the formation of new complex compounds.

In order to find new plant growth regulators that are competitive with existing commercial preparations, the growth-regulating activity of synthesized thiosulfonates derived from sim-triazine and their complex preparations with a rhamnolipid biocomplex was studied using a standard method. A variant of the experiment without treatment with preparations was used as a control. The experiments were repeated three times, and statistical data processing was performed according to Sokolov A.V., with the deviation of the arithmetic mean not exceeding +1.5%.

Watercress and oat seeds were selected as test objects. The results of the study are presented in Tables 1 and 2.

Table 1.

Screening of S-esters of thiosulfonic acids derived from sim-triazine, RBC, and their compositions for growth-regulating activity in relation to watercress

№	Preparation	Test culture (watercress)	
		Similarity, %	Linear root length indicators, % relative to control
			Root
1	RBC (0.01 g/l)	102	104
2	MTS-sym-triazine (0.01 g/l)	98	112
3	MTS-sym-triazine + RBC (0.01 g/l + 0.01 g/l)	108	117
4	MTS-sym-triazine + RBC (0.005 g/l + 0.01 g/l)	104	112
5	MTS-sym-triazine + RBC (0.01 g/l + 0.005 g/l)	98	110
6	ETS-sym-triazine (0.01 g/l)	94	123
7	ETS-sym-triazine + RBC (0.01 g/l + 0.01 g/l)	100	131
8	ETS-sym-triazine + RBC (0.005 g/l + 0.01 g/l)	93	123
9	ETS-sym-triazine + RBC (0.01 g/l + 0.005 g/l)	93	125
10	Terpal (Ristinhibitory Drug) (0.01 g/l)	102	67
11	Ivin (growth stimulant) (0.01 g/l)	95	117

The results of the studies show that the thiosulfonates and RBCs studied, when used independently at concentrations of 0.01 g/l, do not increase the germination of watercress seeds. Among the studied combined preparations, only MTS-sym-triazine + RBC preparations at concentrations of 0.01 g/l + 0.01 g/l and 0.005 g/l + 0.01 g/l are capable of slightly improving germination – by 8 and 4 %, respectively.

All studied compounds and their complex preparations showed the ability to stimulate the growth of watercress roots. ETS-sym-triazine and its complex preparations showed higher stimulating properties than MTS-sym-triazine and complexes based on it. The ETS-sym-triazine + RBC complex in a concentration of 0.01 g/l + 0.01 g/l performed best – it activates watercress root growth by 31%. Among the MTS-sym-triazine + RBC complexes, the preparation with a concentration of 0.01 g/l + 0.01 g/l showed the greatest stimulating properties – it stimulates root growth by 17%.

Table 2.

Screening of S-esters of thiosulfonic acids derived from sim-triazine, RBC, and their compositions for growth-regulating activity in relation to oats

№	Preparation	Test culture (oats)		
		Similarity, %	Linear indices of plant part length, % relative to control	
			Root	Stem
1	RBC (0.01 g/l)	105	113	110
2	MTS-sym-triazine (0.01 g/l)	107	112	114
3	MTS-sym-triazine + RBC (0.01 g/l + 0.01 g/l)	110	126	125
4	MTS-sym-triazine + RBC (0.005 g/l + 0.01 g/l)	112	118	121
5	MTS-sym-triazine + RBC (0.01 g/l + 0.005 g/l)	105	121	120
6	ETS-sym-triazine (0.01 g/l)	110	124	128
7	ETS-sym-triazine + RBC (0.01 g/l + 0.01 g/l)	121	150	157
8	ETS-sym-triazine + RBC (0.005 g/l + 0.01 g/l)	117	134	137
9	ETS-sym-triazine + RBC (0.01 g/l + 0.005 g/l)	119	129	131
10	Terpal (Ristinhibitory Drug) (0.01 g/l)	105	78	98
11	Ivin (growth stimulant) (0.01 g/l)	104	121	111

It has been established that all the compounds studied and their complex preparations increase the germination of oat seeds. Complex preparations of ETS-sym-triazine with RBC proved to be better than ETS-sym-triazine when used alone and better than preparations based on MTS-sym-triazine. The ETS-sym-triazine + RBC complex at a concentration of 0.01 g/l + 0.01 g/l showed the highest stimulating ability, namely, it increased the germination of oat seeds by 21% compared to the control.

The studied RBCs, thiosulfoesters, and their complex preparations showed growth-stimulating activity in relation to the root and stem of oats. The complex preparations of both thiosulfonates with RBC showed a higher growth-stimulating effect than ETS-sym-triazine, MTS-sym-triazine, and RBC when used independently at concentrations of 0.01 g/l. ETS-sym-triazine at a concentration of 0.01 g/l stimulates both root and stem growth of oats more effectively than MTS-sym-triazine at the same concentration. The same pattern can be observed for all complex preparations – complex preparations based on ETS-sym-triazine stimulate the growth of oat roots and stems better than MTS-sym-triazine preparations. It has been established that ETS-sym-triazine and its complex preparations stimulate stem growth slightly better than root growth in oats. The best results were demonstrated by the complex preparation ETS-sym-triazine + RBC at a concentration of 0.01 g/l + 0.01 g/l – it stimulates the growth of oat roots by 50% and stems by 57%.

Among all the studied preparations, the highest growth-stimulating activity against watercress and oats was shown by the complex preparation ETC-sym-triazine + RBC at a concentration of 0.01 g/l + 0.01 g/l. This preparation also has the best effect on germination for oat seeds. Watercress germination indicators increased under the action of the complex preparation MTC-sym-triazine + RBC at a concentration of 0.01 g/l + 0.01 g/l.

As a result of the study, it can be stated that compositions based on synthesized thiosulfonates and rhamnolipid biocomplex of Bacillus spp. and their compositions are effective growth regulators for plants. Based on the UV spectra of solutions of thiosulfonates, rhamnolipid biocomplex and their compositions, the stability of complex preparations over time was investigated.