SECTION 5. PLANT GROWING

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5.1 Effect of physiologically active substances on primary growth processes of winter wheat

Traditional agricultural practices, in particular the intensive use of pesticides and mineral fertilizers in the practical absence of effective antidotes and non-compliance with a number of agronomic measures, in many cases cause deterioration of soil quality, reducing the quantitative and qualitative indicators of yield of cultivated plants. In this case, the links between the components of not only agrophytocenoses, but also ecosystems as a whole are disturbed.

At the same time, the number of genome abnormalities in somatic and germ cells increases, which poses a direct threat to many plant and animal species. Therefore, the problem of reducing the ecological load on the agrophytocenosis is relevant, especially in the arid conditions of the steppe zone of Ukraine [139, 140]. One of the most effective ways of solving this problem is to create a balanced farming system in which plant growth regulators play an important role. Most of the currently recommended preparations are characterized by a narrow spectrum of action [141, 142].

In addition, there is insufficient information about the mechanism of biological action of pesticides, so it is difficult to predict the consequences of their use. Therefore, the development of fundamentally new complex preparations of natural origin is more promising [143]. These preparations can have both herbicidal and stimulating activity depending on the combination of components. In this case, the impact on ecosystems should be minimal.

The use of environmentally safe growth regulators with a wide range of action will increase plant resistance and ensure optimal realization of the varietal (genetic) properties of major crops, as well as obtaining stable harvests [144, 145, 146]. The development of highly effective complex preparations of plant growth regulators will significantly reduce the cost of herbicides and mineral fertilizers, increase the yield and reduce the cost of agricultural products. The ability to predict the physiological state

of plants under the influence of physiologically active substances makes it possible to minimize the negative consequences of adverse weather conditions.

The aim of the work was to study the influence of natural physiologically active substances on the growth processes of winter wheat plants and to develop the most effective complex preparations of plant growth regulators.

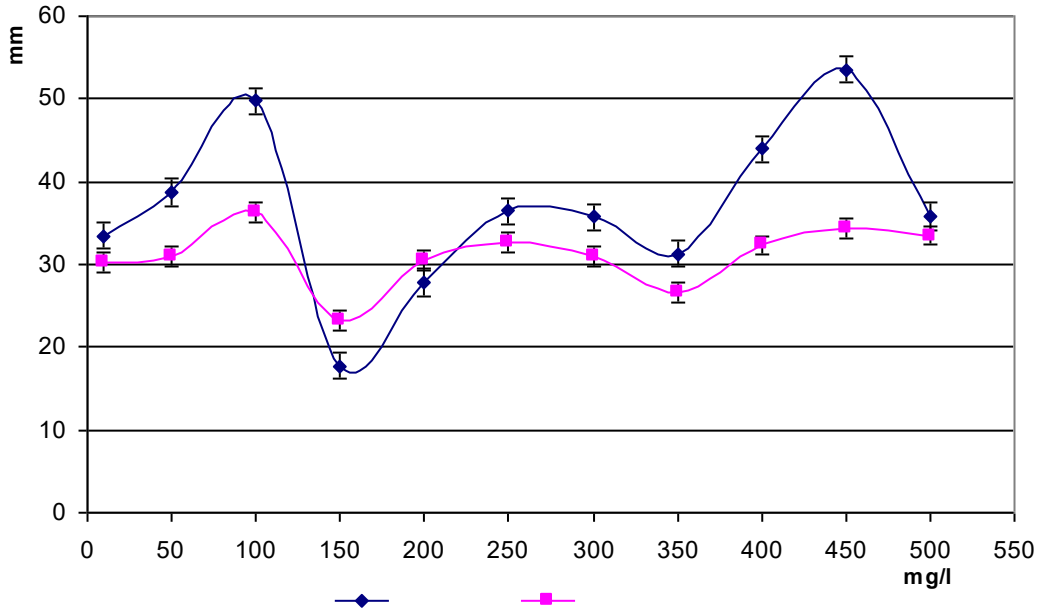
The research object was winter wheat plants of Odessa semi-dwarf variety at the early stages of ontogenesis. Seeds of *Setaria pumila* (Poir.) Roem. & Schult. and common hedgehog *Echinochloa crus-galli* (L.) Beauv. – plants of the family *Poaceae*, which are the most widespread weeds of winter wheat crops.

Sodium humate of lignin origin and its chemical modifications, some amino acids and vitamins as biologically active substances, and sim-triazine herbicides were used to develop complex preparations of growth regulators.

The intensity of primary growth processes of wheat roots and coleoptiles was evaluated as a biotest to study the physiological activity of the preparations [147]. Seedlings were grown in Petri dishes on media with different growth regulators and determined the reaction of seedlings 48, 72 and 96 hours after soaking in solutions with different concentrations of biological active substances.

5.1.1 Plant growth responses to modifications of humus preparations

The physiological activity of sodium humate has been studied in a number of works [148– 153], but up to now the question about the biological effectiveness of the preparations in chemical modification of humic acids remains relevant. In our studies, six modifications of humate were studied in concentrations from 50 to 500 mg/l, with an interval of increasing concentration of 50 mg/l. Dose-effect relationships for different humate modifications after exposure of seedlings for 96 hours are presented below (Fig. 1–5).



Figure

1. Effect of permethylgumate on primary growth processes of winter wheat (row 1 – root length, mm; row 2 – coleoptile length, mm)

Permethylhumate practically did not show growth-stimulating properties in the studied dose intervals, sometimes causing inhibition of growth processes (Fig. 1). The most pronounced negative response to treatment with permethylhumate was observed in growth processes of winter wheat coleoptiles in the concentration range 150–200 mg/l (Fig. 1) with control values of root length 50,0±1,2 mm and coleoptile length 35,3±0,9 mm.

In the variant with paracetylhumate there was no noticeable reaction of growth processes of roots and coleoptiles (Fig. 2). We can note some acceleration of seedlings growth at doses of 50–100 mg/l by 10–13% relative to control, which for the roots was 43,7±1,2 mm, for coleoptiles – 34,8±0,9 mm.

Under the influence of persulfochloride humate, inhibition of growth processes of both the root system and coleoptiles is observed (Fig. 3), given that the control values of root length were 48,0±1,3 mm, coleoptile length – 34,7±0,7 mm. The greatest inhibitory effect is manifested in the concentration range of 200–250 mg/l, and at 400–500 mg/l seedlings practically do not develop.

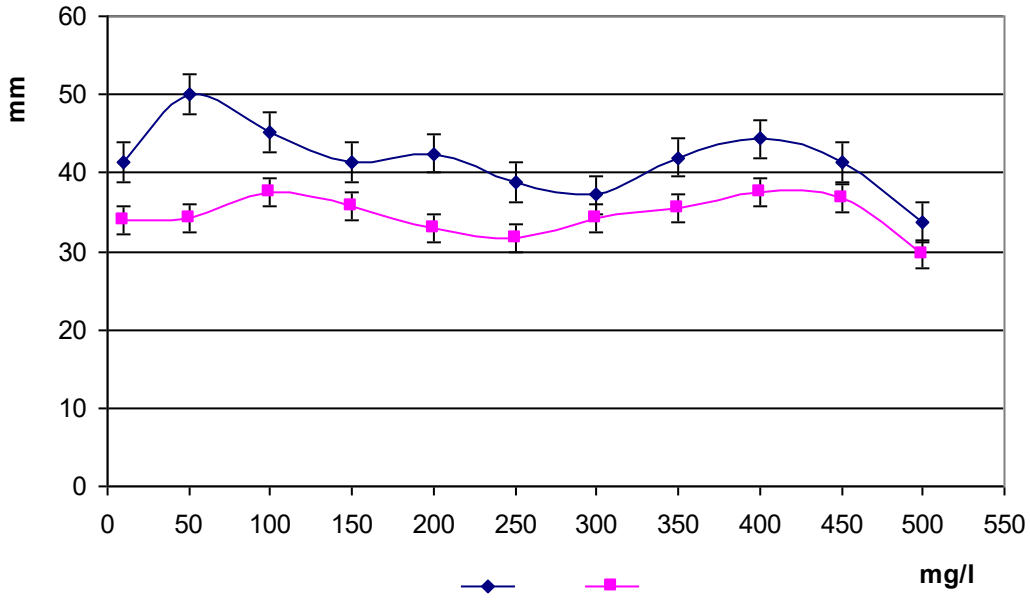


Figure 2. Effect of paracetylgumate on primary growth processes of winter wheat (row 1 – root length, mm; row 2 – coleoptile length, mm)

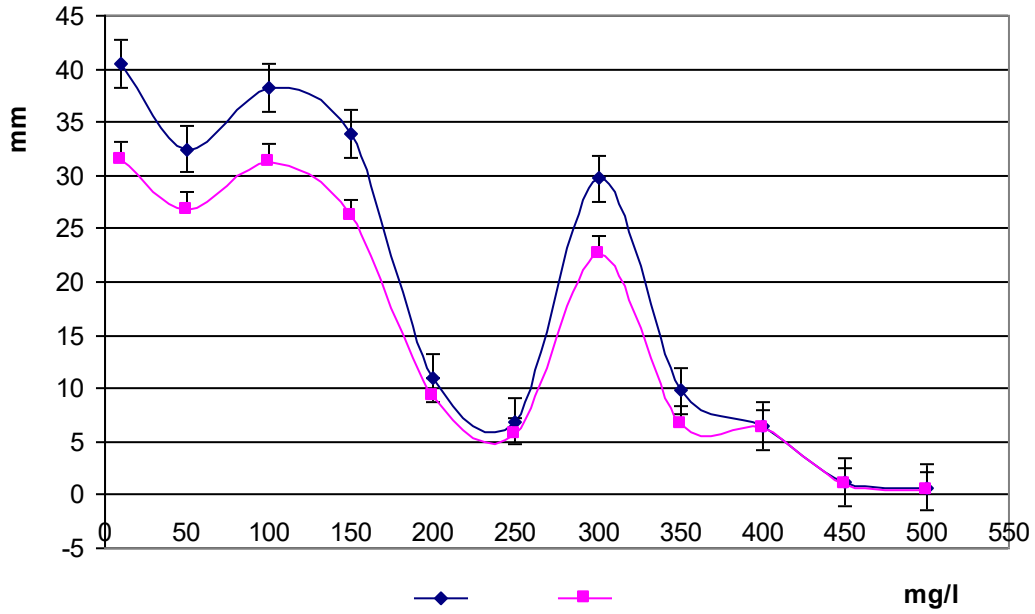


Figure 3. Effect of persulfochloridehumate on the primary growth processes of winter wheat (row 1 – root length, mm; row 2 – coleoptile length, mm)

Under the influence of different concentrations of ferrohumate (except 200 mg/l), the stimulation of growth processes of roots and coleoptiles relative to the control, which in this case was respectively 14,8±0,9 mm and 9,0±0,7 mm (Fig. 4).

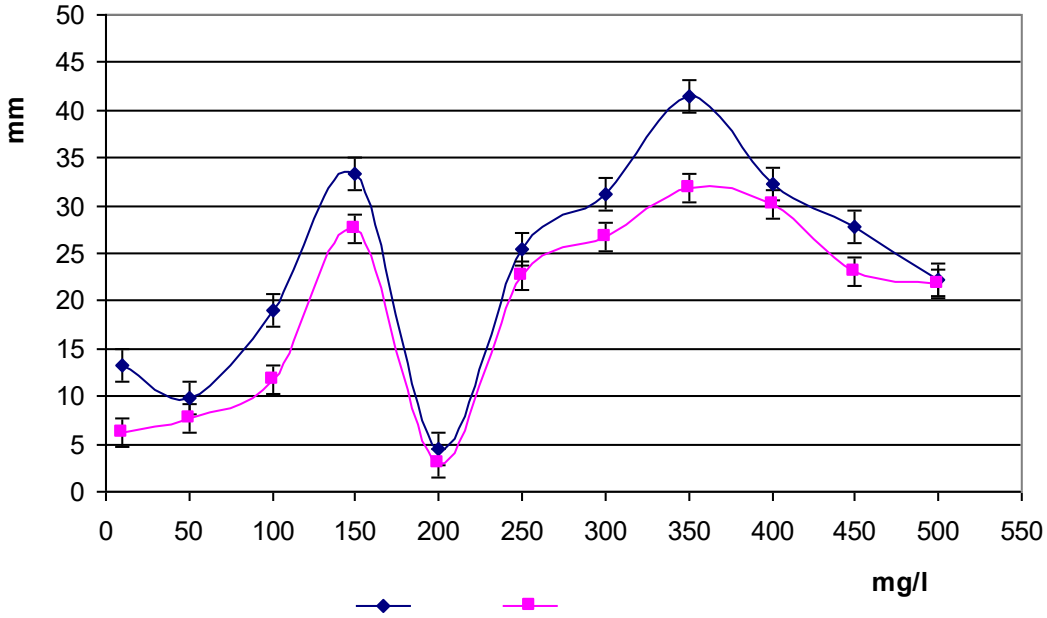


Figure 4. Effect of ferrohumate on primary growth processes of winter wheat (row 1 – root length, mm; row 2 – coleoptile length, mm)

One can note the high physiological activity of ferrohumate in the concentration range of 250–450 mg/l, which stimulates the growth of winter wheat. Root and stem length increased by 1,5–3 times.

Sulfurethanolgumate proved to be almost neutral in effectiveness and had little effect on the primary growth processes of winter wheat (Fig. 5). In the variant with sulfurethanolgumate, the maximum growth of roots and coleoptiles exceeding the control values by 2,5–3 times was observed only at maximum concentrations of the preparation. Taking into account that the length of roots and coleoptiles was 14,2±1,0 mm and 8,8±0,8 mm in the control variant, we may consider stimulating also sulfurethanolgumate concentrations in the range from 150 to 250 mg/l.

For the variant with pernitrozilogumat (Fig. 6) the stimulating concentrations can be considered 50–150 mg/l and 450 mg/l in relation to control, which was 27,5 mm for the roots and 17,0 mg/l for coleoptiles. The growth of winter wheat on the pernitrosylgumate background in the first 72 hours did not differ significantly from the control, but on the fourth day the length of roots and coleoptiles increased significantly – by 40% relative to the control.

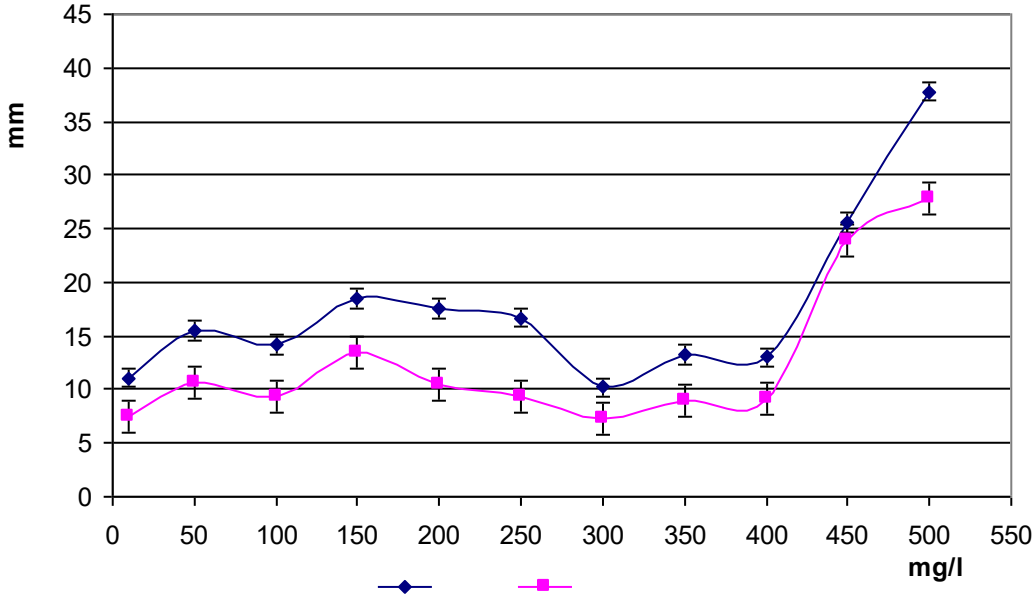


Figure 5. Effect of sulfurethanolgumate on the primary growth processes of winter wheat (row 1 – root length, mm; row 2 – coleoptile length, mm)

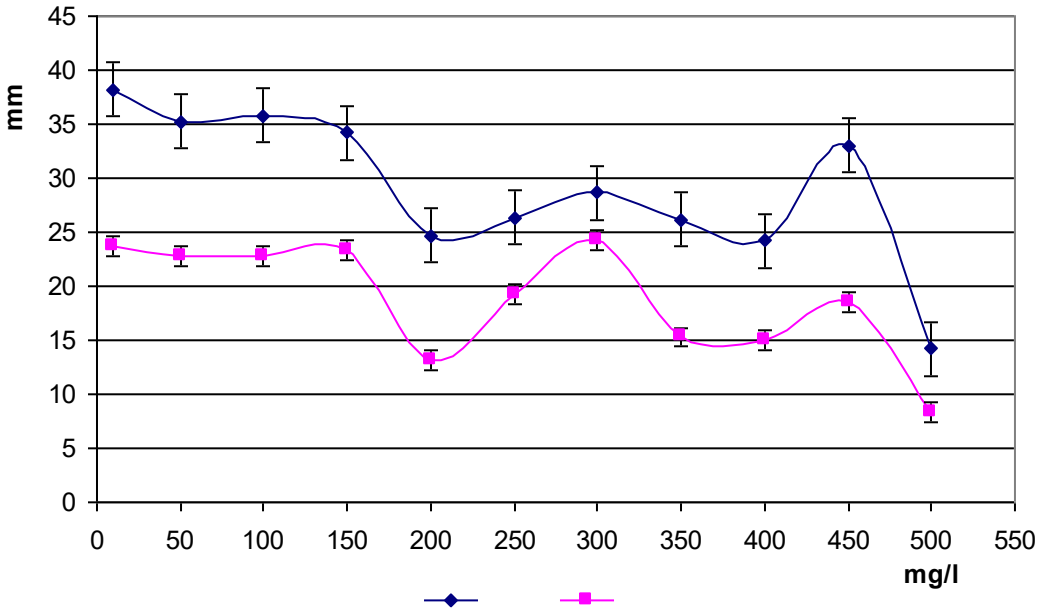


Figure 6. Effect of pernitrosylgumat on the primary growth processes of winter wheat (row 1 – root length, mm; row 2 – coleoptile length, mm)

Thus, based on the studies, we can conclude that permethylhumate and sulfurethanolhumate showed no pronounced physiological activity. Persulfochlorohumate can be used to produce compounds that inhibit plant growth, i.e. compounds with herbicidal properties. Paracetylhumate, ferrohumate, and pernitrosylhumate exhibit growth stimulating properties, so these preparations may be

promising for further research. Various modifications of humic substances allow us to significantly expand the spectrum of biological activity and obtain new plant growth regulators based on natural raw materials.

5.1.2 Study of biological activity of complexes of sodium humate with amino acids

The possibility of using amino acids for regulation of growth processes of cultivated plants has been repeatedly considered in scientific works [154, 155, 156, 157]. In particular, it was shown that dicarboxylic amino acids (asparagine and glutamine acid), their amides (aspragine and glutamine), alkaline amino acids (lysine and arginine), and the iminoacid proline can significantly stimulate root formation in bean seedlings [158, 159]. At the same time, there is evidence that other amino acids had an inhibitory effect or showed no stimulating effect. According to the authors, the additional supply of carbon and nitrogen and formation of indolyl amino acid conjugates, as well as the synthesis of specific proteins and nucleic acids necessary for the induction of rhizogenesis processes by auxins can be the basis for acceleration of growth processes. In addition, the use of amino acids may change the intensity of iron ion absorption, especially against the background of methionine and asparagic acid [160]. However, to date, the possibilities of biological activity of complexes of sodium humate with amino acids on plants of winter wheat have not been studied sufficiently. For this purpose, we studied the complex effect of sodium humate with amino acids in different concentrations on the growth processes of winter wheat.

5.1.2.1 Study of glutamine in the complex of biologically active substances

Based on the results of preliminary studies of different combinations of doses of sodium humate (170 and 220 mg/l) and amino acid glutamine solutions ($2{,}5\cdot10^{-3}$ M and $5\cdot10^{-4}$ M), it was found that the effect of interaction between the two components remained positive throughout the experiment. However, a complete response to the complex effect was observed only for winter wheat roots, whereas for coleoptiles, the growth reactions were not ambiguous. Thus, coleoptile growth during 48 hours did not

depend significantly on the factor studied, after 72 hours the stimulating effect of sodium humate and synergistic effect of the complex components appeared, and after 96 hours they had an additive effect on the optimization parameter, with sodium humate – positively, in glutamine – negatively. In general, it can be considered that the complex of sodium humate and glutamine has a stimulating effect in the early stages of development of winter wheat, and the most effective combinations of sodium humate (170 mg/l and 220 mg/l) and glutamine ($5 \cdot 10^{-4}$M) concentrations were used in further experiments.

Experiments were carried out according to the following scheme: winter wheat seeds were soaked in the complex solutions and after 18 hours some of the seedlings were transplanted to distilled water, and some were transferred to the initial medium. Growth indices were measured 48, 72, and 96 hours after transferring the seedlings to the growth medium. Table 1 shows the results for the time exposure of 48 and 96 hours. At the 72-hour exposure, measurements were carried out only in three experiments, so the results are given below in the text.

Table 1.

Changes in growth responses of winter wheat on the background of of sodium humate and glutamine complex

Vari-ant	Seed germination medium / Germination medium	48 hours		96 hours	
		Root length, mm	Coleoptiles length, mm	Root length, mm	Coleoptiles length, mm
1	2	3	4	5	6
1	Water distilled / Water distilled	6,5 ± 0,52	3,5 ± 0,15	28,0 ± 1,19	29,3 ± 1,07
2	Glutamine $5 \cdot 10^{-4}$M / Water distilled	12,0 ± 0,63*	5,7 ± 0,32*	47,8 ± 2,43*	40,8 ± 1,39*
3	Sodium humate 170 mg/l Water distilled	10,1 ± 1,06*	4,7 ± 0,44	41,3 ± 2,65*	33,2 ± 1,47
4	Sodium humate 170 mg/l + Glutamine $5 \cdot 10^{-4}$M / Water distilled	10,5 ± 0,71*	5,3 ± 0,30*	31,0 ± 2,21*	31,2 ± 2,11

1	2	3	4	5	6
5	Sodium humate 170 mg/l + Glutamine $5 \cdot 10^{-4}$M / Sodium humate 170 mg/l + Glutamine $5 \cdot 10^{-4}$M /	7,2 ± 0,45	4,4 ± 0,23	36,9 ± 1,34*	35,0 ± 1,14*
6	Sodium humate 220 mg/l / Water distilled	8,7 ± 0,78	4,1 ± 0,36	23,8 ± 2,10	23,2 ± 2,20
7	Sodium humate 220 mg/l + Glutamine $5 \cdot 10^{-4}$M / Water distilled	11,9 ± 0,73*	6,2 ± 0,40*	35,1 ± 1,90*	37,0 ± 1,75*
8	Sodium humate 220 mg/l + Glutamine $5 \cdot 10^{-4}$M / Sodium humate 220 mg/l + Glutamine $5 \cdot 10^{-4}$M /	4,1 ± 0,22	3,1 ± 0,18	29,4 ± 1,14	28,5 ± 0,95

Note: * statistically significant difference relative to control at $p < 0.05$

In the variants of experiments with 72-hour exposure the following results were obtained: in the control variant №1 (water/water) the root length was 21,0 ± 0,64 mm, coleoptile length – 14,0 ± 0,82. In treatment № 5 with a solution of sodium humate 170 mg/l and glutamine, the growth indices of roots increased by 20,5% (25,3 ± 0,96 mm), coleoptiles – by 38,6% (19,4 ± 0,81 mm). In treatment № 8, when the concentration of sodium humate is increased to 220 mg/l in the presence of glutamine, the complex has an inhibitory effect on the growth parameters of roots - by 7.6% (19,4 ± 0,74 mm), coleoptiles – by 3,6% (13,5 ± 0,68 mm).

As can be seen from Table 1, in some cases, pre-soaking of seeds with subsequent transplanting of seedlings to water proved to be more effective. Thus, complexes of sodium humate with glutamine effectively stimulate the primary growth processes of winter wheat. The following variants of practical use are possible:

– adding sodium humate 170 mg/l : glutamine $5 \cdot 10^{-4}$ M to the root nutrition medium in a ratio of sodium humate 170 mg/l : glutamine $5 \cdot 10^{-4}$ M;

– soaking the seeds for 18 hours in a solution of sodium humate 220 mg/l: glutamine $5 \cdot 10^{-4}$ M with subsequent cultivation under normal conditions.

This indicates the possibility in principle the practical use of this complex, since the consumption of the necessary components is minimal, and growth stimulation is comparable with the results of the first option – the root length is increased by 35%, coleoptiles – by 30%.

5.1.2.2 Study of methionine in the complex of biologically active substances

Methionine plays an important role in the plant organism in the metabolism of iron, one of the essential components of all cytochromes, catalase, peroxidase [160]. Iron ions in non-heminine form participate in the functioning of the main redox systems of photosynthesis and respiration, which catalyze the initial steps of chlorophyll synthesis (formation of δ-aminolevulinic acid and protoporphyrins). Therefore, when there is an insufficient supply of iron, which is observed under conditions of insufficient moisture, in carbonate soils, there is a decrease in the intensity of respiration and photosynthesis of plants. Studies on maize have shown that the rate of iron ion absorption is highest when the amino acid methionine is present in the growing medium [161]. In cereals, this sulfur-containing amino acid is used to form non-protein amino acids – phytosiderophores, which are intensively released by roots into the environment when plants have insufficient iron supply [162] They are capable of chelating iron ions from poorly soluble inorganic Fe^{3+} compounds. The Fe^{3+} complex with an extracellular non-protein amino acid is transported by a highly specific transport system without iron reduction or dissociation of chelates [163].

In this regard, the practical use of methionine is promising in agricultural crop production. However, the wide application of methionine in this area is limited by technological and economic conditions. A more affordable option may be the use of methionine in combination with other biologically active substances.

The optimal ratio of sodium humate and methionine was established in preliminary experiments, which were carried out according to the scheme of factor experiment planning. It was found that the response of winter wheat seedlings to the complex of sodium humate with methionine varies depending on the stage of development, and the growth of roots and coleoptiles depends on the studied

physiologically active substances. The most optimal results were obtained when using a complex of sodium humate 220 mg/l and methionine $7,5 \cdot 10^{-5}$ M. The data obtained in the experiments to assess the effectiveness of this complex at exposures of 48 and 72 hours are presented in Table 2, and at exposures of 48 hours – in Fig. 7.

As can be seen from Table 2, a certain acceleration of growth processes in winter wheat throughout the experiment is observed against the background of the studied complex.

Table 2.

Changes in growth responses of winter wheat on the background of of sodium humate and methionine complex

Vari-ant	Seed germination medium / Germination medium	48 hours		96 hours	
		Root length, mm	Coleoptiles length, mm	Root length, mm	Coleoptiles length, mm
1	Water distilled / Water distilled	4,2 ± 0,37	3,2 ± 0,11	18,2 ± 0,75	9,7 ± 0,74
2	Methionine $7,5 \cdot 10^{-5}$ M / Water distilled	5,4 ± 0,43*	4,0 ± 0,23 *	18,0 ± 0,80	12,5 ± 0,63*
3	Sodium humate 220 mg/l / Water distilled	5,3 ± 0,46	3,5 ± 0,21	19,0 ± 0,71	10,8 ± 0,68
4	Sodium humate 220 mg/l + Methionine $7,5 \cdot 10^{-5}$ M / Water distilled	6,8 ± 0,55*	3,9 ± 0,26	20,0 ± 0,91	13,0 ± 0,60*
5	Sodium humate 220 mg/l + Methionine $7,5 \cdot 10^{-5}$ M / Sodium humate 220 mg/l + Methionine $7,5 \cdot 10^{-5}$ M	11,6 ± 0,74*	7,9 ± 0,59*	30,2 ± 1,33*	22,6 ± 0,85*

Note: * statistically significant difference relative to control at $p < 0.05$

Root growth in relation to the control was 190–350%, coleoptile growth was 190–260%. Maximum stimulation of growth processes is observed in the first 48 hours of seedling development, which is especially evident with the combined effect of both components – sodium humate and methionine. The effect of enhancing the growth

processes remains even after 96 hours of growing seedlings on a complex solution of these components (Fig. 7).

It was found that constant cultivation on medium containing sodium humate and methionine complex is more effective than soaking the seeds in this solution for 18 hours and further transplanting to distilled water. However, even in the latter case the growth stimulation is quite noticeable, because in this variant of the experiment the growth indicators of roots exceed the control values by 15–90%, coleoptiles – by 15–50%.

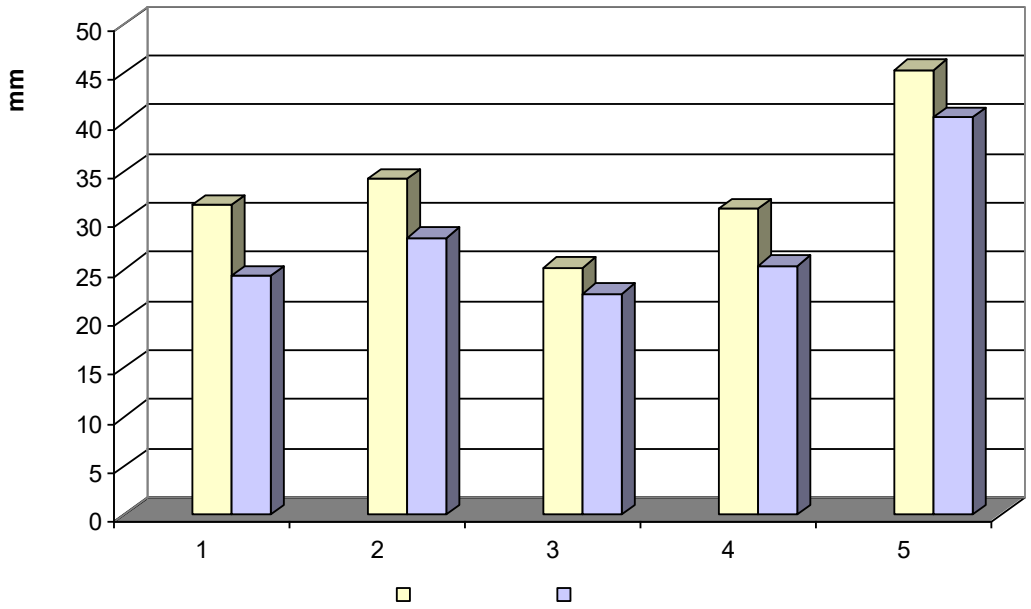


Figure 7. Growth responses of seedlings after 96 h exposure to sodium humate solutions sodium humate and methionine (row 1 – root length; row 2 – coleoptile length). *Note:* variants 1-5 in the figure correspond to variants 1-5 in Table 2.

The data obtained suggest the possibility of practical use of sodium humate 220 mg/l in combination with methionine $5 \cdot 10^{-5}$ M by soaking the seeds for 18 hours as the most technologically and economically affordable approach to solving the problem of activation of growth processes of winter wheat in the early stages of ontogenesis.

5.1.2.3 Study of arginine in the complex of biologically active substances

The presence of amine and guanidine groups in the molecule determines the basic (alkaline) properties of arginine. Arginine as a proteinogenic amino acid and some of its derivatives non-proteinogenic amino acids (ornithine, canavanine) in the plant organism are accumulated in germinating seeds. This indicates the important role of these amino acids in the metabolism of germinating seeds, as their content drops sharply during seed germination [164]. In this regard, the importance of studying the effect of arginine as a component of a complex preparation on the growth processes of cultivated plant seedlings is obvious.

Preliminary studies on the physiological activity of the complexes of sodium humate with arginine have shown that the greatest efficiency is observed in the range of concentrations of sodium humate 170–220 mg/l and arginine from $2{,}5 \cdot 10^{-3}$ M to $1 \cdot 10^{-5}$ M. In order to clarify the concentrations of the components, the experiment on factor analysis of the dependence of growth processes of winter wheat on each of the components and on the complex as a whole was carried out. Based on the analysis of mathematical models a combination of sodium humate 220 mg/l and arginine $1 \cdot 10^{-4}$M was chosen as the most effective complex.

The data obtained in the experiments to assess the effectiveness of this complex at exposures of 48 and 72 hours are presented in Table 3, and in Fig. 8, at 48 hours exposure.

Table 3.

Changes in growth responses of winter wheat on the background of of sodium humate and arginine complex

Vari-ant	Seed germination medium / Germination medium	48 hours		96 hours	
		Root length, mm	Coleoptiles length, mm	Root length, mm	Coleoptiles length, mm
1	2	3	4	5	6
1	Water distilled / Water distilled	4,2 ± 0,34	3,2 ± 0,19	18,2 ± 0,72	9,7 ± 0,70
2	Arginine $1 \cdot 10^{-4}$M / Water distilled	4,9 ± 0,58	3,8 ± 0,25	15,7 ± 1,10	9,7 ± 0,82

1	2	3	4	5	6
3	Sodium humate 220 mg/l / Water distilled	5,3 ± 0,42*	3,5 ± 0,22	19,0 ± 0,77	10,8 ± 0,69
4	Sodium humate 220 mg/l + Arginine $1 \cdot 10^{-4}$M / Water distilled	7,1 ± 0,57*	4,1 ± 0,23*	22,0 ± 0,86*	11,7 ± 0,84*
5	Sodium humate 220 mg/l + Arginine $1 \cdot 10^{-4}$M / Sodium humate 220 mg/l + Arginine $1 \cdot 10^{-4}$M	7,6 ± 0,52*	5,0 ± 0,18*	30,8 ± 1,14*	19,9 ± 1,68*

Note: * statistically significant difference relative to control at $p < 0.05$

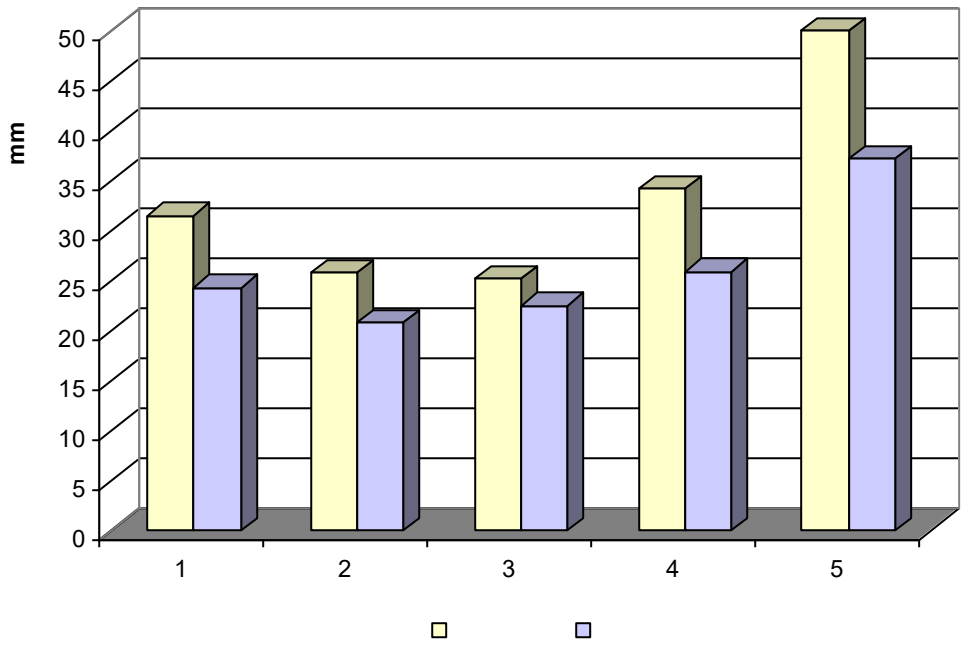


Figure 8. Growth responses of seedlings after 96 h exposure to sodium humate and arginine solutions (row 1 – root length; row 2 – root length). *Note*: variants 1-5 in the figure correspond to variants 1-5 in table 3.

The results show that the greatest stimulation of growth processes of winter wheat was observed against a complex of physiologically active substances in the growing medium. In treatment № 5 after 48 h the linear growth of roots and coleoptiles was 155% and 156%, in 72 h – 169% and 205% respectively. The tendency to

stimulation of growth processes was retained after 96 h: the length of roots and coleoptiles was 164% and 153%, respectively, compared to the control (Fig. 8).

It should be noted that high efficiency was also noted in the variant № 4 with soaking the seeds in sodium humate solution with arginine for 18 hours and subsequent cultivation in distilled water. In this variant, the root length varied in the range 130–200%, the coleoptile length – 125–130% in relation to the control.

5.1.2.4 Study of lysine in the complex of biologically active substances

Lysine, like arginine, belongs to the diaminomonic carboxylic acids that are, on the one hand, a source of amine nitrogen in the metabolic reactions of plants and, on the other hand, acceptors of ammonium nitrogen in protein hydrolysis as a result of natural causes or under the action of stress factors.

Preliminary experiments on the plant response to the combination of sodium humate and lysine showed that in the concentration range 170–220 mg/l and $2{,}5 \cdot 10^{-4}$ M – $7{,}5 \cdot 10^{-4}$ M, respectively, these components exhibit high biological activity. Selection of the most optimal combination was carried out by a planned factorial experiment, which established the most effective concentrations of the components for their complex effect on the seedlings: 170 mg/l of sodium humate solution and $7{,}5 \cdot 10^{-4}$ M lysine solution. The results of studying the complex action of these preparations on the growth processes of winter wheat after 48 and 72 h of exposure are presented in Table 4, and after 96 h of exposure – in Fig. 9.

Table 4.

Changes in growth responses of winter wheat on the background of of sodium humate and lysine complex

Vari-ant	Seed germination medium / Germination medium	48 hours		96 hours	
		Root length, mm	Coleoptiles length, mm	Root length, mm	Coleoptiles length, mm
1	2	3	4	5	6
1	Water distilled / Water distilled	4,2 ± 0,38	3,2 ± 0,17	18,2 ± 0,75	9,7 ± 0,73

1	2	3	4	5	6
2	Lysine $7,5 \cdot 10^{-4}$ M / Water distilled	5,9 ± 0,56*	4,1 ± 0,24*	15,3 ± 0,91*	10,3 ± 0,72
3	Sodium humate 220 mg/l / Water distilled	5,3 ± 0,45	3,2 ± 0,20	19,0 ± 0,74	10,8 ± 0,67
4	Sodium humate 220 mg/l + Lysine $7,5 \cdot 10^{-4}$ M / Water distilled	6,0 ± 0,55*	4,0 ± 0,23	18,6 ± 1,07	11,0 ± 0,79*
5	Sodium humate 220 mg/l + Lysine $7,5 \cdot 10^{-4}$ M / Sodium humate 220 mg/l + Lysine $7,5 \cdot 10^{-4}$ M	11,1 ± 0,58*	6,1 ± 0,46*	27,4 ± 0,80*	20,5 ± 0,64*

Note: * statistically significant difference relative to control at $p < 0.05$

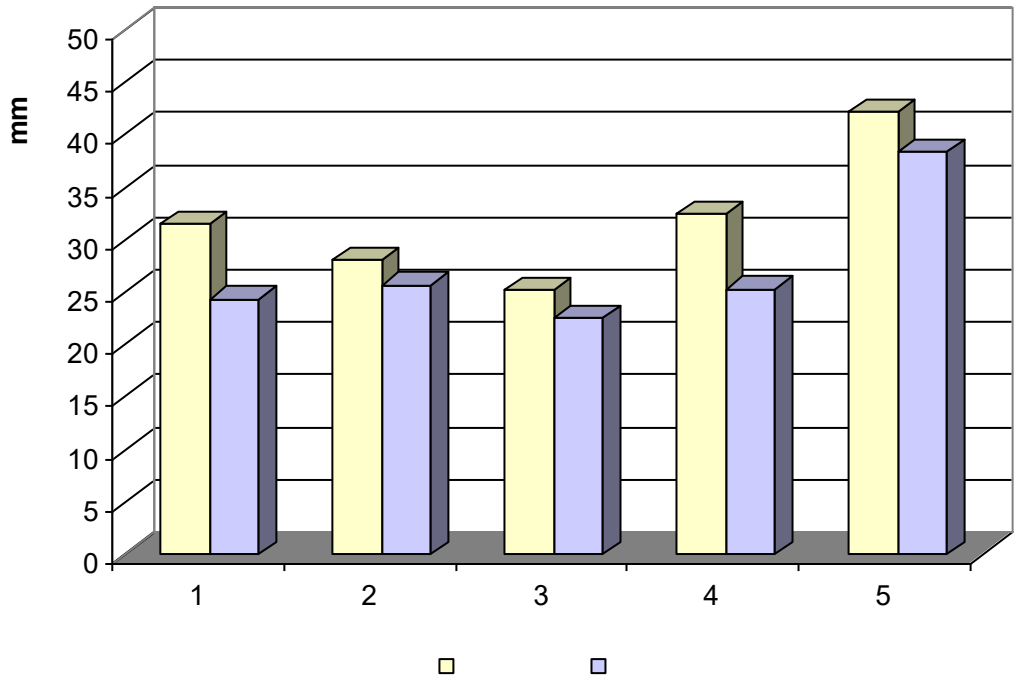


Figure 9. Growth responses of seedlings after 96 h exposure to sodium humate and lysine solutions (row 1 – root length; row 2 – root length). *Note:* variants 1-5 in the figure correspond to variants 1-5 in table 4.

The results show that the studied combination of concentrations of biologically active components of sodium humate and lysine complex was quite effective stimulator

of primary growth processes of winter wheat. Thus, against the background of this complex, root growth was 150–270% of the values in the control variant, coleoptile growth was 175–190%.

Somewhat less stimulation was observed when the seeds were soaked in the solution of the complex of biologically active components with subsequent transplanting to distilled water. But even in this case, the growth of roots exceeded the control values by 16–60%, the growth of coleoptiles – by 18–50%, which gives a reason to recommend the practical use of this complex in agricultural crop production.

5.1.2.5 Study of cysteine in the complex of biologically active substances

The basis for inclusion of cysteine in the program of studies of complex preparations of sodium humate with amino acids was the information about high biological activity of this amino acid containing sulfur, especially in extreme conditions [165]. Preliminary studies have established the concentration range of increased biological activity of the components, which was for sodium humate from 170 to 200 mg/l, for cysteine from $5 \cdot 10^{-5}$ M to $5 \cdot 10^{-4}$ M.

The results of studying the complex action of these preparations on the growth processes of winter wheat after 48 and 72 hours of exposure are presented in Table 5 and in Fig. 10 after 96 hours of exposure.

Table 5.

Changes in growth responses of winter wheat on the background of of sodium humate and cysteine complex

Vari-ant	Seed germination medium / Germination medium	48 hours		96 hours	
		Root length, mm	Coleoptiles length, mm	Root length, mm	Coleoptiles length, mm
1	2	3	4	5	6
1	Water distilled / Water distilled	6,8 ± 0,54	4,3 ± 0,22	22,9 ± 0,81	13,8 ± 0,85
2	Cysteine $5 \cdot 10^{-5}$ M / Water distilled	14,2 ± 1,08*	5,8 ± 0,35	37,2 ± 1,73*	23,8 ± 1,10*

1	2	3	4	5	6
3	Sodium humate 170 mg/l / Water distilled	15,3 ± 0,65*	7,0 ± 0,58*	37,8 ± 1,46*	24,2 ± 0,82*
4	Sodium humate 170 mg/l + Cysteine $5 \cdot 10^{-5}$ M / Water distilled	15,1 ± 0,85*	5,9 ± 0,34	35,3 ± 1,52*	23,8 ± 0,76*
5	Sodium humate 170 mg/l + Cysteine $5 \cdot 10^{-5}$ M / Sodium humate 170 mg/l + Cysteine $5 \cdot 10^{-5}$ M	6,3 ± 0,46	4,3 ± 0,29	22,9 ± 1,07	14,0 ± 0,85
6	Sodium humate 220 mg/l / Water distilled	13,5 ± 0,64*	6,2 ± 0,46*	41,3 ± 1,25*	24,3 ± 0,69*
7	Sodium humate 220 mg/l + Cysteine $5 \cdot 10^{-5}$ M / Water distilled	14,1 ± 0,73*	6,1 ± 0,48*	38,3 ± 1,47*	24,5 ± 0,83*
8	Sodium humate 220 mg/l + Cysteine $5 \cdot 10^{-5}$ M / Sodium humate 220 mg/l + Cysteine $5 \cdot 10^{-5}$ M	6,5 ± 0,55	4,4 ± 0,20	25,7 ± 0,91	17,8 ± 0,75

Note: * statistically significant difference relative to control at $p < 0.05$

The study of plant growth responses to the action of this complex showed that the stimulation of growth processes of winter wheat averaged 133–210% for roots, for coleoptiles 137–150%.

It should be noted that at the exposition of 48 hours the greatest efficiency of the complex of cysteine solutions with sodium humate at a concentration of 170 mg/l was observed, at the exposition of 72 hours the complex with sodium humate at a concentration of 220 mg/l showed a greater biological activity. At the 96-hour exposure, the activity of sodium humate at 220 mg/l as a part of the complex with cysteine was also slightly higher compared with the 170 mg/l concentration.

In general, soaking the seeds for 18 h in a solution of sodium humate complex with cysteine and subsequent transplantation to distilled water was even slightly more effective than in the variants with the presence of components of the complex in the growing medium. Based on these results, it is possible to recommend the practical use

of the complex preparation of sodium humate in combination with cysteine ($5 \cdot 10^{-5}$ M) for stimulation of the primary growth processes of winter wheat.

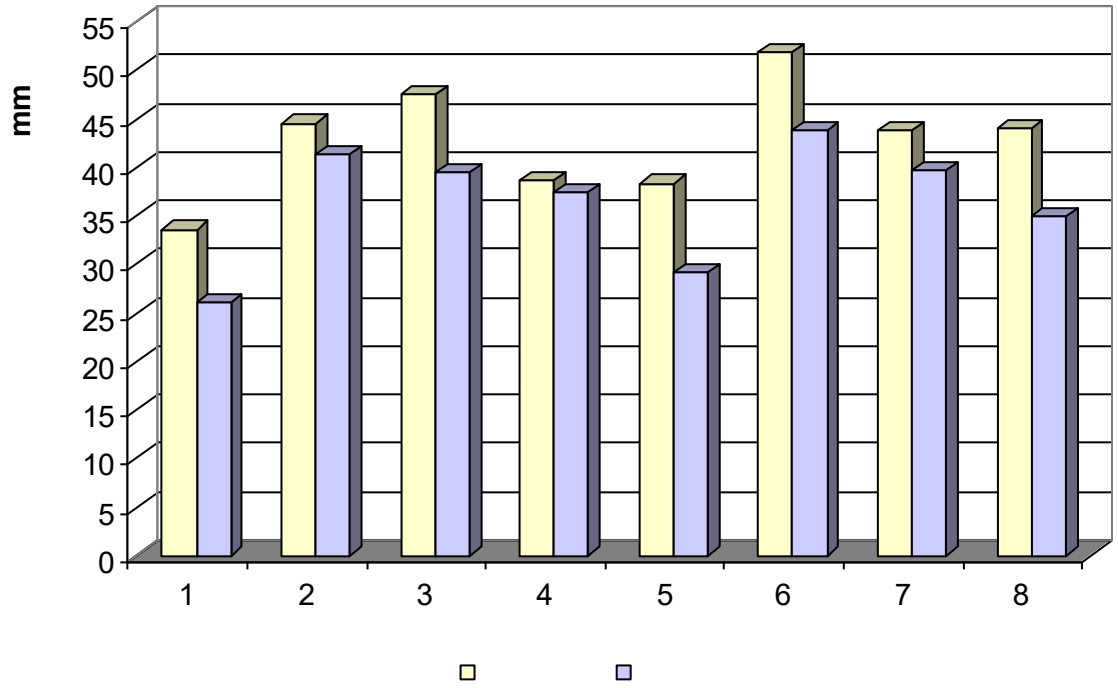


Figure 10. Growth responses of seedlings after 96 h exposure to sodium humate and cysteine solutions (row 1 – root length; row 2 – root length). *Note:* variants 1-8 in the figure correspond to variants 1-8 in table 5.

Thus, as a result of the research aimed at obtaining new biologically active preparations based on sodium humate and a number of amino acids, the most effective combinations and concentration ranges of components which stimulate the growth processes of winter wheat at the early stages of ontogenesis have been established:

- sodium humate 170 and 220 mg/l + glutamine $5 \cdot 10^{-4}$ M
- Sodium humate 220 mg/l + methionine $7{,}5 \cdot 10^{-5}$ M
- Sodium humate 220 mg/l + arginine $1 \cdot 10^{-4}$ M
- sodium humate 170 mg/l + lysine $7{,}5 \cdot 10^{-4}$ M
- Sodium humate 170 and 220 mg/l + cysteine $5 \cdot 10^{-5}$ M

Different variants of using the obtained combinations of physiologically active substances have been considered. Particularly, it was shown that soaking the seeds for 18 hours in the solutions of the preparations complex is almost as efficient as soaking them, and in some cases is even superior to variants with constant presence of the

preparations in growing medium. Therefore, it is possible to recommend the agrotechnical method of seed pre-soaking in solutions of physiologically active substances to stimulate the primary growth processes of winter wheat.

5.1.3 Study of biological activity of complexes of sodium humate with vitamins

The use of water-soluble vitamins to regulate the growth processes of plants has been studied in many works [166, 167, 168], although to date, this problem has not been sufficiently developed. A number of studies on this issue indicate the possibility of using some vitamins to influence various processes of plant morphogenesis, growth and development, including yield components [169]. It was found that ascorbic acid can induce plant nitrate reductase activity, as well as reduce the inhibitory effect of herbicides. B vitamins at concentrations of $1{,}9 \cdot 10^{-3}$ M (vitamin B_1), $2{,}6 \cdot 10^{-4}$ M (vitamin B_2) and $2{,}9 \cdot 10^{-3}$ M (vitamin B_6) contribute to restoration of plant cell ultrastructure by activating the degradation of 2,4-D conjugates with cellular components. Data on the physiological activity of vitamins used exogenously suggest the possibility of their use as one of the components for obtaining complex preparations.

Vitamin B_2 (riboflavin) is a member of the active group of redox enzymes involved in hydrogen transfer in various metabolic processes of the plant organism. Therefore, the introduction of this biologically active component into the growing medium can have a stimulating effect on the development of plants in the early stages of ontogenesis. After carrying out preliminary experiments, the approximate concentration range (from 150 to 400 mg/l) in which high biological activity of sodium humate and vitamin B_2 is possible when used together was determined.

To study the dose dependence of winter wheat growth, a series of experiments were carried out according to the following scheme. In variant 1 – distilled water (control). In the following variants the ratios of sodium humate and vitamin B_2 were: 2 – 150 and 150 mg/l; 3 – 150 and 400 mg/l; 4 – 50 and 150 mg/l; 5 – 50 and 400 mg/l; 6 – 100 and 275 mg/l; 7 – 400 and 250 mg/l; 8 – 400 and 270 mg/l; 9 – 300 and 250 mg/l; 10 – 300 and 270 mg/l; 11 – 350 and 260 mg/l.

The results of the study of dose dependence of growth processes in winter wheat for 48, 72 and 96 hours showed that the complex of biologically active substances in different concentrations has a stimulating effect mainly on the root growth of seedlings. The greatest effect is achieved at 96 h exposure in almost all variants of the experiment (Fig. 11).

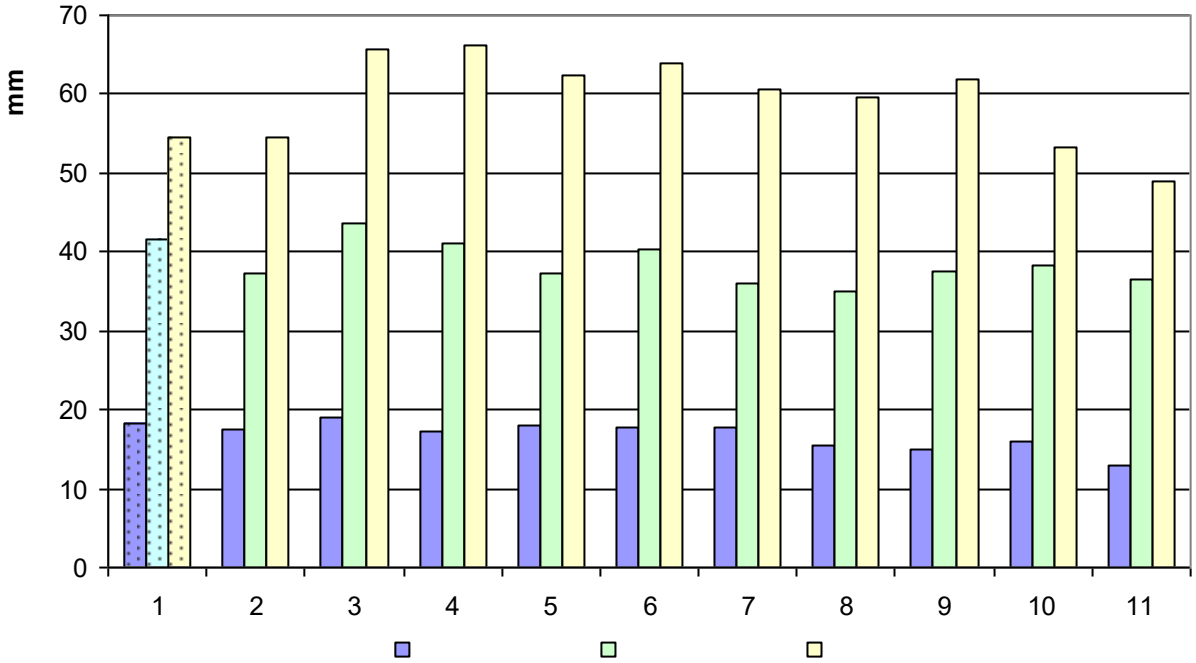


Figure 11. Dose dependence of winter wheat root growth after 48 h (row 1), 72 h (row 2), and 96 h (row 3) on solutions of sodium humate and vitamin B_2 solutions. *Note:* variants of experiments 1-11 are described in the text.

Thus, quite acceptable ratios of doses of sodium humate and vitamin B_2 that stimulate root growth processes by 96 hours of seedling development were revealed. In the first approximation, these compositions can be considered as regulating the growth of plants and can be used for pre-sowing treatment of seeds. Complex formulations with sodium humate at a concentration of 150–200 mg/l and vitamin B_2 at a concentration of 50–100 mg/l may be more promising.

Vitamin C (ascorbic acid) is an important component of the antioxidant defense of the plant organism. The participation of ascorbic acid in redox processes occurring at the cellular level is due to the fact that vitamin C can easily change from oxidized to reduced form and vice versa. Interconversions of ascorbic and dehydroascorbic acids

in the plant organism are closely related to the enzymatic interactions of oxidized and reduced glutathione, thus forming the plant antioxidant defense system [170, 171]. Therefore, introducing this biologically active component into the growing medium can have a stimulating effect on plant development and increase their resistance to adverse factors already in the early stages of ontogenesis. After conducting preliminary experiments, an approximate range of concentrations was determined - for sodium humate 300–500 mg/ml, for vitamin C 150–250 mg/l, in which high biological activity of sodium humate and vitamin C is possible with their combined use.

To study the dose dependence of winter wheat growth, a series of experiments were carried out according to the following scheme. In variant 1 – distilled water (control). In the following variants the ratios of sodium humate and vitamin C were: 2 – 350 and 250 mg/l; 3 – 300 and 250 mg/l; 4 – 350 and 200 mg/l; 5 – 300 and 200 mg/l; 6 – 325 and 225 mg/l; 7 – 500 and 200 mg/l; 8 – 400 and 200 mg/l; 9 – 500 and 150 mg/l; 10 – 400 and 150 mg/l; 11 – 450 and 175 mg/l.

The results of the dose-dependent study of growth processes in winter wheat for 48, 72 and 96 hours showed that the complex of sodium humate and vitamin C has a stimulating effect on coleoptile growth processes and, to a greater extent, on root growth of seedlings (Fig. 12).

It was found that the greatest positive effect of the complex effect of biologically active substances is manifested only after 96 hours of germination.

Of all the options for combining the concentrations of the two components, the most optimal ratios of sodium humate and vitamin C in the composition of the complex can be identified. Presumably, the active combinations are sodium humate 300–350 mg/l and vitamin C 200–250 mg/l. In this case, after 96 hours of exposure an increase in root length by 15–25% was observed.

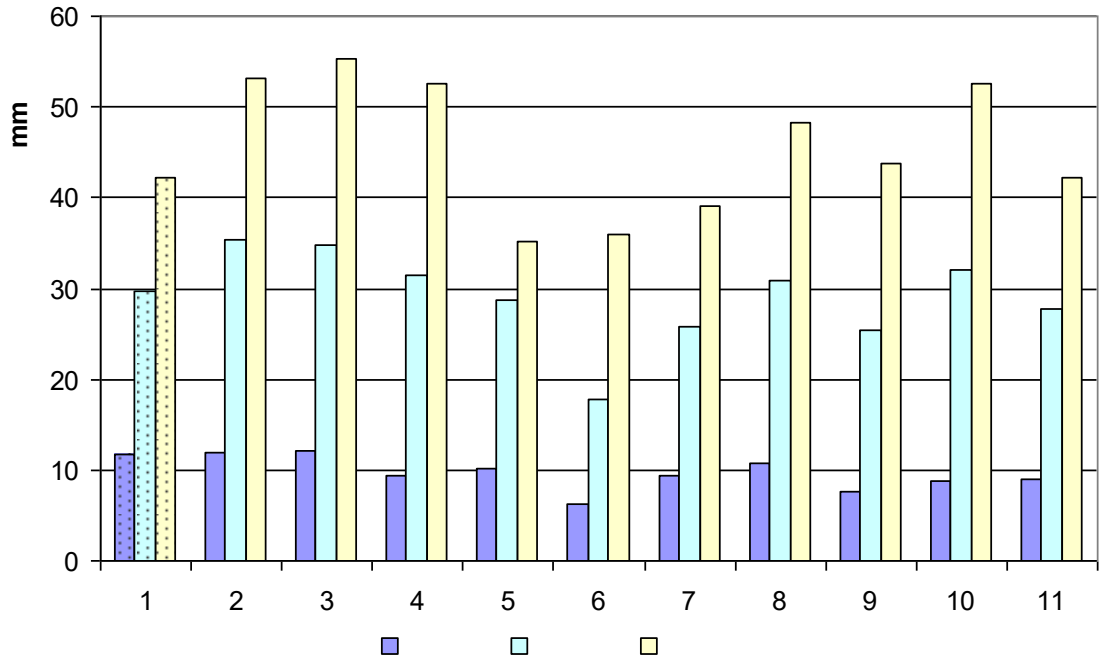


Figure 12. Dose dependence of winter wheat root growth after 48 h (row 1), 72 h (row 2), and 96 h (row 3) on sodium humate and vitamin C solutions. *Note*: variants of experiments 1-11 are described in the text.

Thus, according to the results of the study, it can be concluded that the complexes of sodium humate with vitamins B_2 and C allow obtaining biologically active preparations, the effectiveness of which exceeds the positive effects of each component separately. Based on the analysis of empirical and statistical regularities, the ranges of concentrations of sodium humate and vitamins which give the most optimal combinations of components in the complex and have a stimulating effect on the primary growth reactions of winter wheat at the early stages of development have been revealed.

5.1.4 Study of biological activity of complexes of sodium humate with symtriazine half-life products

The use of herbicides assumes no inhibitory effect of these drugs on the growth of cultivated plants. For a long time, symtriazines remained one of the most common chemical means of weed control, including in winter wheat [142, 172, 173]. However,

the effect of symtriazine group herbicides, and mainly their decomposition products, on cultivated plants cannot be completely excluded [174].

For this purpose, we conducted a series of experiments to study the physiological activity of symtriazine group herbicides and their half-life products on winter wheat plants, using doses of drugs equivalent to doses of herbicides applied in the field conditions [175, 176] (Table 6, Fig. 13).

Table 6

Growth responses of winter wheat plants to symtriazines and their half-life products

Vari-ant	Seed germination medium / Germination medium	48 hours		72 hours	
		Root length, mm	Coleoptiles length, mm	Root length, mm	Coleoptiles length, mm
1	Water distilled (control)	7,5 ± 0,57	5,0 ± 0,14	26,3 ± 1,35	16,8 ± 1,12
2	Atrazine 200 mg/l	7,3 ± 0,57	4,1 ± 0,25*	26,2 ± 1,17	13,5 ± 1,08*
3	Atrazine 250 mg/l	7,1 ± 0,56	4,6 ± 0,29*	24,3 ± 0,64	15,3 ± 0,72
4	Simazine 160 mg/l	6,9 ± 0,53	4,3 ± 0,24*	24,2 ± 0,86	14,5 ± 0,61*
5	Simazine 250 mg/l	7,7 ± 0,50	4,6 ± 0,23*	24,8 ± 0,78	16,2 ± 0,79
6	2-chloro-4-isopropylamino-6-amino-symmtriazine 100 mg/l	7,3 ± 0,55	5,0 ± 0,16	22,3 ± 0,74*	15,5 ± 0,77
7	2-chloro-4-isopropylamino-6-amino-symmtriazine 150 mg/l	6,0 ± 0,41*	4,4 ± 0,25*	22,3 ± 0,67*	14,5 ± 0,82*
8	2-Chloro-4-ethylamino-6-amino-symmtriazine 50 mg/l	6,8 ± 0,22	4,4 ± 0,23*	20,3 ± 0,82*	14,0 ± 0,88*
9	2-Chloro-4-ethylamino-6-amino-symmtriazine 70 mg/l	7,2 ± 0,48	5,0 ± 0,10	22,8 ± 0,69*	15,7 ± 0,75*

Note: * statistically significant difference relative to control at $p < 0.05$

As follows from the data obtained, atrazine, simazine and their two metabolites proved to be slightly toxic for the root growth of 48-h sprouts, but a statistically insignificant tendency to a decrease in the root and coleoptile growth rate in the presence of these drugs was noted. The exception was 2-chloro-4-isopropylamino-6-

amino-symmtriazine at a concentration of 150 mg/l in the presence of which a statistically significant decrease in root and coleoptile length of winter wheat seedlings was observed. Similar regularities were observed during 72-h exposure of seedlings to solutions of the sim-triazine group, only for simazine (160 mg/l) and 2-chloro-4-ethylamino-6-amino-symmtriazine (50 mg/l) the inhibiting effect was the most stable. Root and coleoptile growth intensity, which at the initial stages of the experiment did not differ significantly from the control, decreased after 96-hour exposure in all experimental variants (Fig. 13).

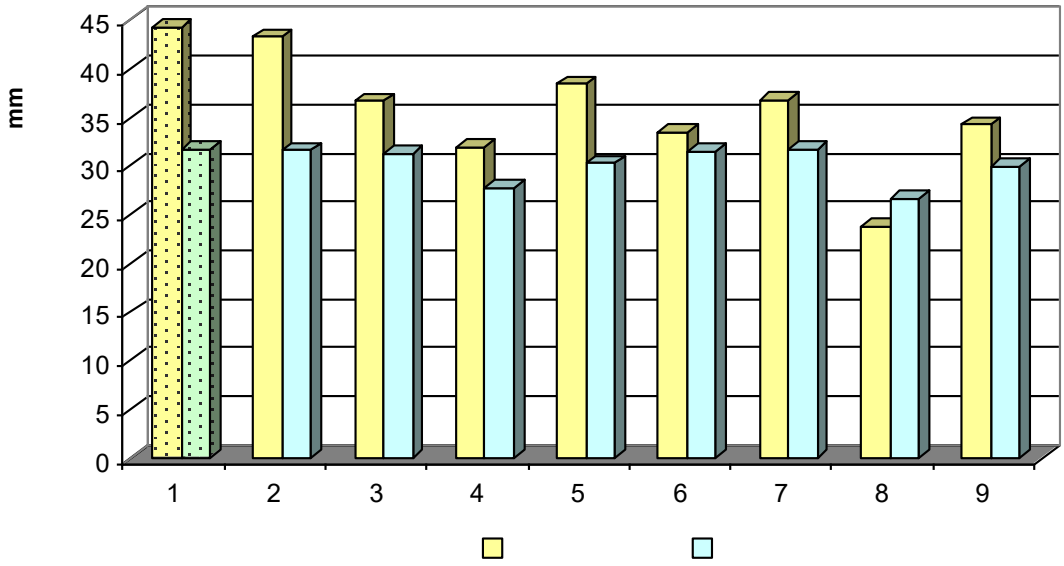


Figure 13. Growth reactions of winter wheat after 96 h exposure on solutions of sym-triazine group preparations (row 1 – root length; row 2 – coleoptile length). *Note:* variants 1-9 in the figure correspond to variants 1-9 in Table 6.

The greatest toxic effect is produced by half-life products of symtriazine group preparations, 2-chloro-4-isopropylamino-6-amino-symtriazine 100 mg/l, 2-chloro-4-ethylamino-6-amino-symtriazine 50 and 70 mg/l on root growth.

Based on the results obtained, we can conclude that symtriazines and their half-life products have a similar effect on the primary growth processes of winter wheat, exhibiting the greatest phytotoxicity on the 4th day of germination. Root length, which is practically unchanged against the background of the studied preparations at the beginning of seedling development, decreases significantly with time. Coleoptile

growth initially decreases in the presence of sim-triazines, but later somewhat stabilized, i.e. there is a change in the susceptibility to herbicides of organs and stages of development of cultivated plants.

In the system of intensive technologies of cultivation of agricultural plants, the problem of herbicides aftereffect and the degree of preservation of physiological (herbicidal) activity not only on crops, but also on weeds remains an important issue. It is known [174] that the herbicides of the sim-triazine group are characterized by a long aftereffect, and therefore, it is necessary to study the half-life products of sim-triazines for their herbicidal properties.

Preliminary investigations allowed us to determine the concentration range of simtriazines and their metabolites, in which a fairly pronounced inhibitory effect on weeds is observed. Experiments on the growth responses of such cereal weeds as *Echinochloa crus-galli* (L.) Beauv. and *Setaria pumila* (Poir.) Roem. & Schult. The choice of these representatives of weeds is explained by the fact that these species are the most widespread in the crops [177, 178, 179, 180].

In the experiments (Fig. 14 and 15) the following concentrations of studied preparations were used: atrazine 200 mg/l (variant 2) and 250 mg/l (variant 3); simazine 160 mg/l (variant 4), 200 mg/l (variant 5) and 250 mg/l (variant 6); 2-chloro-4-isopropylamino-6-amino-simmtriazine 100 mg/l (variant 7) and 150 mg/l (variant 8); 2-chloro-4-ethylamino-6-amino-simmtriazine 50 mg/l (variant 9), 70 mg/l (variant 10) and 100 mg/l (variant 11). Control plants were grown on distilled water (variant 1). The results obtained according to these experimental variants for *Setaria pumila* plants are shown in Fig. 14, for *Echinochloa crus-galli* plants in Fig. 15.

As follows from the results of the studies presented in Fig. 14*a*, the initial dose loads of atrazine and two metabolites give a certain stimulating effect on the growth processes of the weed Setaria pumila, while no such effect was observed for simazine at the earlier stages of development. When the dose load of 2-chloro-4-ethylamino-6-amino-simtriazine increased from 50 to 70 and 100 mg/ml, a sharp inhibition of growth processes occurred.

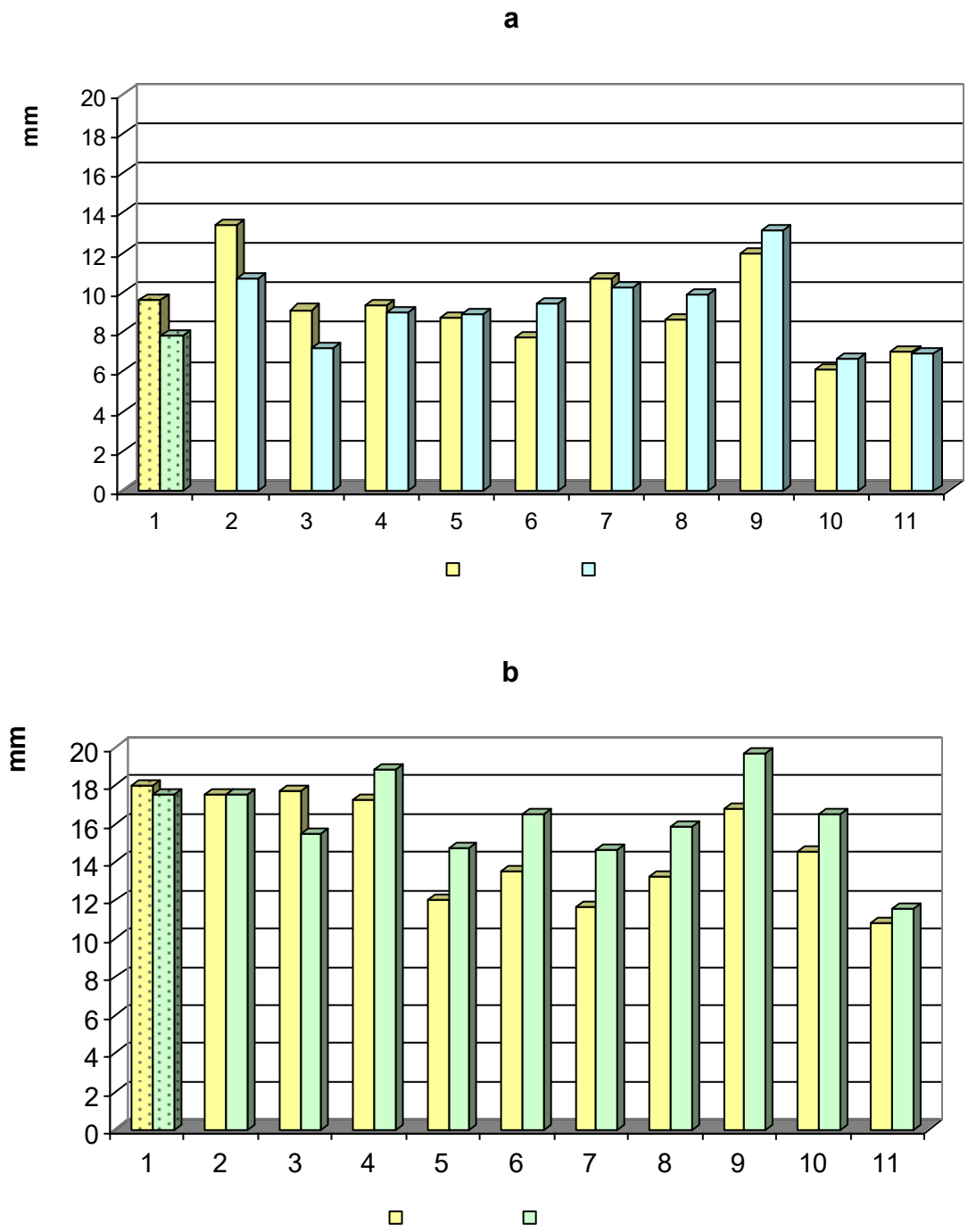


Figure 14. Growth reactions of 6-day-old (a) and 9-day-old (b) seedlings *Setaria pumila* against the background of symmtriazines and their metabolites (row 1 – root length; row 2 – coleoptile length). *Note:* variants 1-11 are described in the text.

The same patterns with respect to this metabolite are also observed in 9-day-old seedlings (Fig. 14*b*). The results show that the initial growth stimulation reactions in 9-day-old seedlings are replaced by growth inhibition. In general, the inhibitory effect

of symtriazines and their metabolites on 9-day-old seedlings is manifested primarily in the reduction of root growth.

The effect of metabolites was manifested in the reduction of the intensity of *Setaria pumila* growth processes against the background of doses that were more than three times lower than the doses of atrazine and simazine. Sustained inhibition of development was observed at the concentration of symtriazine half-life products of 50 mg/l, while a similar effect, and only for root growth, was observed against a background of atrazine 200 mg/l and simazine 160 mg/l. Among the studied metabolites, 2-chloro-4-ethylamino-6-amino-symmtriazine was characterized by increased toxicity for weeds, as the decrease in the intensity of Setaria pumila growth processes was observed already at the initial stages of development.

All the studied compounds turned out to be unfavorable for the growth of *Echinochloa crus-galli* already at the initial stages of development. Root length was inhibited more significantly than the growth of the aboveground mass (Fig. 15). However, a similar inhibitory effect was achieved at significantly lower doses of the metabolites than the initial sim-triazines.

It is possible to assume the great efficiency of using the herbicidal properties of 2-chloro-4-isopropylamino-6-amino-symmtriazine and 2-chloro-4-ethylamino-6-amino-symmtriazine in winter wheat cultivation to control weeds.

It is also possible to expand the range of symmtriazine effectiveness and modify their biological activity by obtaining complexes of these herbicides with the products of their half-life. The mixture consisting of 50 mg/l of atrazine with different doses of 2-chloro-4-isopropylamino-6-amino-symmtriazine (10–50 mg/l) and 2-chloro-4-ethylamino-6-amino-symmtriazine (10–50 mg/l) had a detrimental phytotoxic effect on *Setaria pumila* plants. Such results allow us to consider the use of the mixture of this composition to control this weed more effective than the previously studied solutions of atrazine, simazine and their metabolites. This aspect is all the more important because it was found that *Setaria pumila* plants in the early stages of development show relative resistance to herbicides of the symtriazine group, unlike *Echinochloa crus-galli* plants.

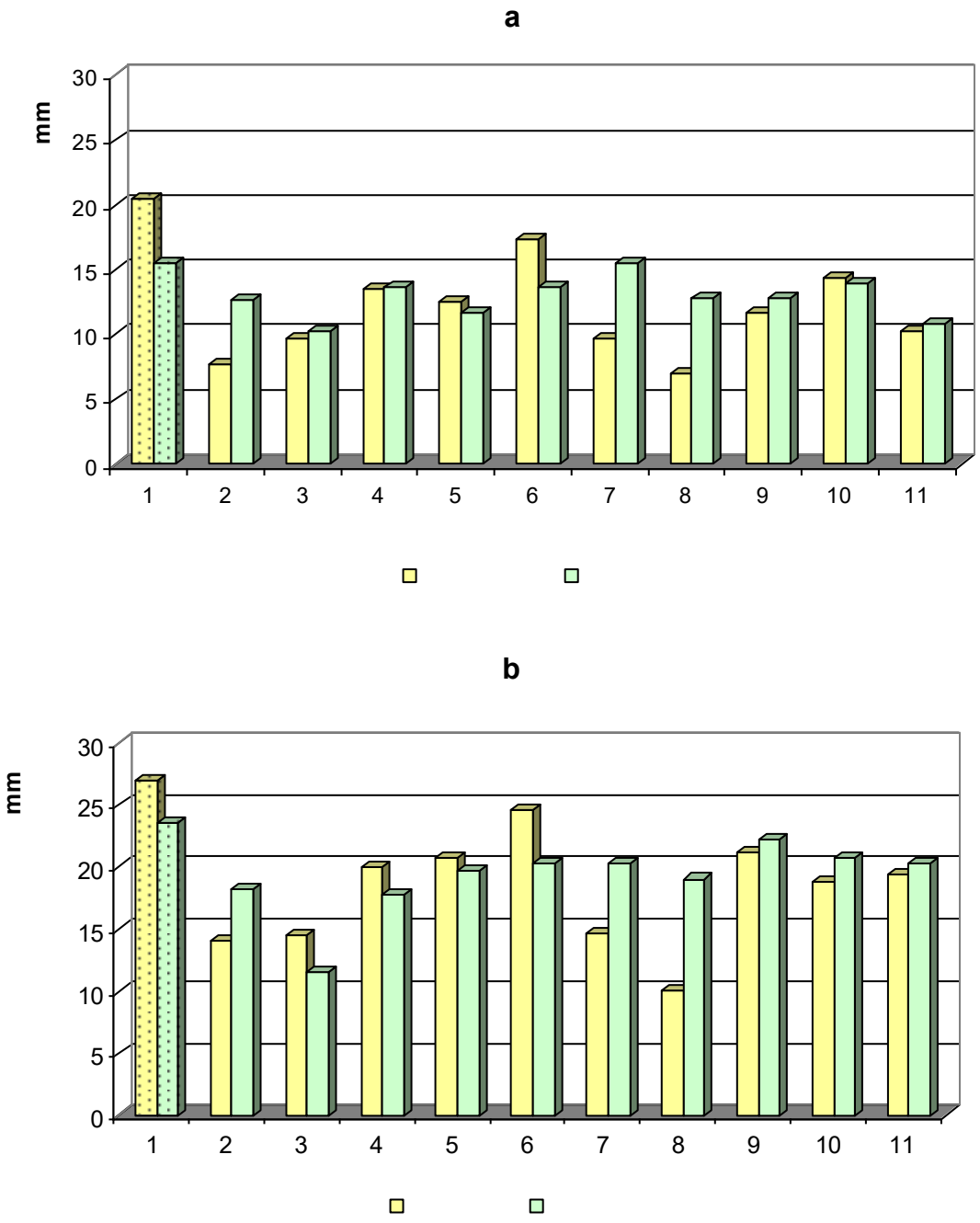


Figure 15. Growth reactions of 6-day-old (a) and 9-day-old (b) seedlings *Echinochloa crus-galli* against the background of symmtriazines and their metabolites (row 1 – root length; row 2 – coleoptile length). *Note:* variants 1-11 are described in the text.

As a result of testing the effectiveness of mixtures of atrazine with its half-life products against *Echinochloa crus-galli*, we found that pronounced inhibitory activity was characteristic of the mixture consisting of atrazine (50 mg/l) and 2-chloro-4-ethylamino-6-amino-symmetriazine (10 mg/l). Other combinations of these drugs in

different doses showed no increased phytotoxic activity on the growth processes of this weed.

In contrast, the complexation of atrazine with different doses of 2-chloro-4-isopropylamino-6-amino-symmtriazine leads to a significant inhibition of the growth processes of *Echinochloa crus-galli* already at the first stages of development. The following mixture composition can be considered the most optimal: atrazine 50 mg/l and 2-chloro-4-isopropylamino-6-amino-symmtriazine 10 mg/l. In this case, minimization of the amount of chemicals in the composition of the complex is achieved and inhibition of growth processes of the weed *Echinochloa crus-galli* is expressed strongly enough.

Thus, based on the results of the study of the biological activity of complexes of sodium humate with the products of symmtriazines half-life, we can conclude. Simtriazines are able to inhibit the growth of cultivated plants in doses of 150–200 mg/l. Since the tissue effect is combined with the high stability of these drugs in the soil, their use is not rational from an environmental point of view.

It was found that symtriazine half-life products can inhibit the growth and development of such common weeds as *Setaria pumila* and *Echinochloa crus-galli* more effectively and at lower doses than the initial herbicides. Mixtures of atrazine (50 mg/l) with 2-chloro-4-isopropylamino-6-amino-symmtriazine (10–50 mg/l) as well as with 2-chloro-4-ethylamino-6-amino-symmtriazine (10–50 mg/l) completely stop the development of *Setaria pumila*. To suppress the primary growth processes of *Echinochloa crus-galli*, combinations of atrazine at a dose of 50 mg/l with 2-chloro-4-ethylamino-6-amino-symmetriazine (10 mg/l) and 2-chloro-4-isopropylamino-6-amino-symmetriazine (10-50 mg/l) are very effective.

Conclusion

The results of studies have shown that by chemical modification of sodium humate it is possible to obtain compounds with growth-regulating properties (paracetylhumate, ferrohumate, pernitrosylhumate) as well as compounds inhibiting plant growth, i.e. having herbicidal properties (persulfochloridehumate). The ranges of concentrations providing the highest physiological activity of these compounds on the

primary growth processes in winter wheat have been established experimentally and this broadens the range of growth regulators on the basis of natural raw materials.

It has been shown, that the complex preparations on the basis of sodium humate and amino acids in a certain range of concentrations have a stimulating effect on the primary growth processes of winter wheat. It has been found that the physiological effect of complex preparations of sodium humate with vitamins B_2, C exceeds the biological activity of the initial components.

It has been established that symtriazine metabolites possess more expressed phytotoxic effect in relation to weed plants in comparison with initial herbicides and their complex formulations with simazine and atrazine are effective inhibitors of cereal weeds growth processes at winter wheat crops. The combination of simtriazines with the products of their decomposition allows to obtain fairly effective inhibitors of growth processes of cereal weeds, while reducing the rates of application, which allows to consider them more environmentally acceptable drugs than the original herbicidal compounds.