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USE OF BIOLOGICALLY ACTIVE SUBSTANCES IN AGRICULTURAL PREPARATIONS

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Abstract. Introduction. *The production and use of growth stimulants, both as single preparations and in combination with traditional organo-mineral fertilizers, is of great scientific interest and practical significance. In this connection, one of the most promising approaches to solving this problem is the development of preparations based on plant growth regulators, including both biostimulants obtained by microbiological synthesis and biostimulants obtained by chemical synthesis.*

Purpose. *To develop a growth-stimulating preparation using non-traditional raw materials and test it in laboratory and field conditions on pilot batches of barley and wheat seeds encapsulated with the preparation being studied, which contained the following components: sodium humate, hydrated fullerenes, an aqueous solution of shungite, microbiological carotene in a certain ratio.*

Methods. *Laboratory and field experiments to study the effect of the application of preparations with different components on the growth and development of barley and spring wheat were carried out on the basis of V. Dokuchaev Kharkiv National Agrarian University at the Department of Plant Growing in 2020.*

Results. *The results obtained during laboratory experiments showed that the application of the preparation "Humir" had a stimulating effect on the growth of plants. Seedlings in the test variant appeared a day earlier than the control ones, and the plants of the test variant were ahead of the control ones in development. At the end, the average length of seedlings was 9.5 cm in the control, and it was 13.8 cm in the test. The average mass of seedlings was 1.6 g in the test. Thus, in the preliminary*

laboratory experiments it has been shown that the application of the preparation at the first stages of plant growth had a stimulating effect, ensuring an increase in the length and mass of seedlings in the tests compared to the control ones.

Discussion. *Research and development of new non-traditional preparations which, along with known plant growth stimulants, increase the productivity of crops in food production were carried out. The developed preparation "Humir", in addition to increasing the yield of grain crops, had good wettability and a film-forming effect. After spraying, it caused a creation of thin film on the surface of the leaf, which was being absorbed by the plant for several days. Because of this, "Humir" can also be successfully used to combat diseases such as powdery mildew, late blight, bacteriosis, etc.*

Key words: *growth stimulants, fullerenes, shungite, humates, carotene, biologically active substances*

Introduction. At nowadays a significant number of scientific publications and patents, both domestic and foreign, indicate a great interest and practical significance in the production and application of growth-stimulating preparations, both as single preparations and in combination with traditional organo-mineral fertilizers (in the form of bioadditives for plant growth). This makes the development of biologically active preparations for plant growth an important scientific and technical task. In this connection, one of the most promising approaches to solving this problem is the development of preparations based on plant growth regulators, including both biostimulants obtained by microbiological synthesis and biostimulants obtained by chemical synthesis (Garrett Owen & Whipker, 2019).

An optimal recipe and technology for obtaining and application encapsulated seeds based on the consumption of sequentially supplied biogenic nutrients by plants from the multilayer shell surrounding the seed during the growing season has been developed. The method has been tested on grain seeds, including barley and spring wheat seeds, as well as on medicinal plant seeds (safflower). Wherein it was assumed that the bioadditives containing plant growth stimulants (regulators) had to be a notable impact on an intensifying plant growth.

Analysis of recent researches and publications. The most important direction of research activity of scientists and specialists in the field of agronomy is the searching and development of alternative methods of growing crops that could increase plant productivity and the quality of agricultural products without increasing the rates of fertilizer application and other means of chemicalization of agriculture (Ankita, &Debasish, 2017; Karamany, Sadak, Bakry, 2019). It is caused by the fact that the intensification of agricultural production by introducing significant doses of fertilizers does not lead to an

adequate increase in crop yields, and the application of pesticides increases the toxicity of the soil and changes the chemical composition of the crop and the content of vitamins, enzymes and other substances in the products (Ageguehu & Taye, 2003). Thus, search for new non-traditional compounds that could increase the productivity of crops in food production is a topical issue.

These are the new generation growth regulators, the so-called fertilizers based on humic acids and more. There is a lot of information in the scientific literature about the positive effect of humic substances and preparations developed on their basis on plants (Chen, Clapp, Magen, 2004). The positive effect of humic substances on the growth, development and productivity of agricultural crops there was noted.

Purpose. To develop a growth-stimulating preparation using non-traditional raw materials and test it in laboratory and field conditions on pilot batches of barley and wheat seeds encapsulated with the preparation being studied, which contained the following components: sodium humate, hydrated fullerenes, an aqueous solution of shungite, microbiological carotene in a certain ratio.

Methods. Laboratory and field experiments to study the effect of the application of preparations with different components on the growth and development of barley and spring wheat were carried out on the basis of V. Dokuchaev Kharkiv National Agrarian University at the Department of Plant Growing in 2020.

In laboratory conditions, seeds were germinated in Petri dishes and in rolls. A layer of cotton wool was placed at the bottom of the Petri dish, which was covered with gauze on top. Seeds were placed on the resulting pad. Nutrient solutions were poured under the pad (90 mL of nutrient solution per cup). This technique made it possible as following: 1) to prevent rapid evaporation of solutions, and therefore an increase in the concentration of nutrients in them; 2) the seeds were on the surface, and not in the thickness of the solution; 3) plant roots easily penetrated the cotton pad and strengthened there.

In the first stage of seed germination, Petri dishes were covered with lids on top, leaving a small hole for air penetration. When the first seedlings appeared, the covers were removed.

In the test variants, the plants were grown using a 0.1% solution of all being studied components (the optimal concentration of the solution was optimised in previous experiments), and tap water was used in the control variants. During the entire growing period, the air temperature was maintained at 21-23°C during the day and 17-18°C at night.

In the experiments, plant growth was monitored daily, the number and length of seedlings were measured, and the yield was also recorded (the green mass of the plants was cut and weighed).

The control was the germination of untreated seeds (dry control), the test samples being studied were seeds treated only with a solution of hydrated fullerenes (Baati et al., 2012; Dawid, Górny, Gburski, 2015, shungite water, the preparation "Vympel", a growth regulator based on humic substances, the preparation "Baikal", a biological product with the effective microorganisms, and the preparations being developed "Humir", which consisted of sodium humate, hydrated fullerenes, an aqueous solution of shungite in a certain ratio, and "Humir-1" with microbiological carotene added additionally to the components listed above.

Solutions of preparations being studied were prepared both using hydrated fullerenes and shungites (the distilled water was treated with shungites for 3-5 days). Crushing of shungite up to nanoparticle sizes was carried out by the cryogenic method followed by the measurement of the particle size using a laser scattering analyzer HORIBA LA-910 (Germany). The size of the nanoparticles was 40–50 nm. The number of microelements in the new solution was determined by X-ray fluorescence analysis using ELVAX X-Ray Fluorescence Spectrometer with computer processing of the obtained results.

Results and their discussion. Since the optimal plant density is one of the most important conditions determining the productivity of sowing, both the density of seedlings and the percentage of germinated seeds were determined in laboratory and field conditions (Table 1).

1. Density of harvest of spring wheat 2020, average indicators (550 units = 100%)

Variant	Density of seedlings, units/m^2	Percentage of germinated seeds, %
Dry control	400.8±41.7	72.8±7.9
Water with hydrated fullerene	406.2±42.5	76.5±8.5
Vympel	416.5±43.4	75.6±8.4
Baikal	437.3±45.6	79.5±8.7
Humir	450.9±44.2	81.9±8.0

Based on the experimental data obtained, the effectiveness criteria of the developed bioadditives were calculated and the effectiveness of their application was compared with similar preparations “Baikal” and “Vympel”.

Fullerenes were chosen in this study to examine their effect on the stability of solutions with biologically active substances in comparison with carbon-containing water infused on shungite crushed to nanosize.

The introduction of hydrated fullerenes into the composition of the functional preparation produced in its greater resistance to microbiological contamination (Table 2).

2. Effectiveness of antimicrobial properties of fullerene-like structures in the samples of the functional preparation being studied

Microorganisms	Microbial load after inoculation, lg CFU/mL	The initial microbial load reduction lg			
		Hydrated fullerenes		Shungites	
		2 days	7 days	2 days	7 days
Staphylococcus aureus	5.00	1.2	1.8	1.6	2.5
Pseudomonas aeruginosa	4.99	3.15	3.5	4.02	4.30
Candida albicans	4.98	2.37	3.58	2.85	4.08

As evidenced by the results presented in Table 2, the colony-forming units of all microorganisms being tested shown varying degrees of decrease in the values in 7 days after inoculation when subjected by the samples of preparations being studied. This was determined by the antimicrobial action of hydrated fullerenes and shungite water, the last was also a fullerene-like structure that provided a self-preserving effect. Wherein, fullerenes caused higher antimicrobial properties against the most widespread variations of microbes.

The results of the study of absorbing properties of shungites was given in the work (Jun et al., 2018). Issues related to the interaction of fullerenes with additives added to water-soluble preparations have been remained unresolved however. The reason for this may be objective difficulties associated with the method of detecting fullerenes in solutions, including additives. The expense part in the experiment performing is large, which makes the corresponding studies impractical. The method of detecting fullerenes in the preparations can be an option to overcome the relevant difficulties. This approach is used in the study presented here. Such method development will make it possible not only to determine the quantitative values of fullerenes, but also to substantiate the safety of the solutions being studied when using them.

Concentrated aqueous solutions of hydrated fullerene C60, abbreviated as C60FWS, are molecular-colloidal systems of spherical fractal clusters, wherein the structural unit is a strong, highly hydrophilic supramolecular complex consisting of a C60 fullerene molecule enclosed in the first hydration shell, which contains 24 water molecules (24 – hydrated C60 fullerene (C60HyFn)). The consistently first closely bound hydration shell is

maintained by subsequent ordered water shells.

The size of C60HyFn corresponds to 1.6–1.8 nm. Clusters (secondary associates) of C60HyFn are formed by the fusion of their hydration shells. The sizes of such spherical clusters correspond to a number of values: 3.4; 7.1; 10.9; 14.5; 18.1; 21.8; 25.4; 28.8; 32.4; 36.0 nm.

Hydrated fullerenes create in their environment an ordered structurally heterogeneous aqueous environment, in which the direction and kinetics of chemical and biochemical processes differ from those occurring in pure (disordered) water (Kong & Zepp, 2021; Amjad Bashir et al., 2021). Aqueous solutions of hydrated fullerene C60 have a long universal list of properties even positive biological activity. Therefore, the development of experimental methods for the determination of hydrated fullerenes in solution with biological additives is a topical issue. Acoustic emission (AE) was studied using a modern acoustic-emission complex A-Line32 for determination of the amount of fullerenes in the solution. This method was used to prove the existence of structural formations in water with hydrated fullerenes and to study the dynamics of their transformations. Acoustic emission is easily recorded when dissolving in water various salts, mixing homogeneous liquids, melting ice, proceeding chemical reactions and other similar processes (Rupal, Raval, Saraf, 2020; Teboho, Malebe, Tugizimana, 2022).

As can be seen from the data in the Table 3, the interaction of homogeneous liquids, for example, when adding 0.4 mL of different molecular state water to distilled water, is accompanied by a characteristic, strictly specific for each substance, acoustic emission (AE). This allows fruitful use of AE signals for the study of various physical and chemical processes.

3. Acoustic emission parameters when adding 0.4 mL of various water additives with different molecular structure to water

AE parameters	Water additives		
	Distilled water	Ice/melt water	Solution of hydrated fullerenes
Acoustic pulse generation duration	1.5	89	150
Sum of impulses	6	150	300
AE activity, number of impulses	up to 6	up to 200	up to 400
Amplitude, dB	35	94.5	324.5
Energy, dB	71	85	98
Signal duration, μs	557	up to 5000	up to 10000
Rise time, μs	102	95	84

The appearance of AE signals in melt water and a solution with hydrated fullerenes can only be explained by structural rearrangements in them.

When using C60FWS, the recommended concentration range of hydrated fullerene C60 in the final product corresponds as follows: in water-containing products (drinking water, water for preparing food products, alcoholic drinks, soft drinks, etc.) 0.01-10 μg/L.

When treating spring wheat seeds with the preparation "Humir", which contained fullerenes or shungite water, an increase in the number of both plants per 1 m^2 and the number of stems collected per 1 m^2 was observed. The indicators of the tillering coefficient and the mass of dry plants also exceeded those when treating seeds with the preparation “Humir”.

Photosynthesis is the general function and main process of nutrition of plants as autotrophic organisms. On this basis, to increase the productivity of plants, first of all, it is increase their photosynthetic productivity, formation of a photosynthetic apparatus that is optimal in size and duration of operation and determined mainly by the area of leaves. Treatment of plants with a growth-regulating preparation in the tillering (in spring) contributed to an increase in area leaves of wheat (Table 4).

4. Spring wheat 2020, earing phase, leaf area

Variant	Σ 3^x leaves per 1 shoot, cm^2	The number of shoots per 1 m^2		Leaf area per 1 m^2		Leaf area per 1 hectare, m^2	
		test	control	test	control	test	control
Water with hydrated fullerene	22.66	298.64	218.64	0.67	0.49	6767.8	6017.3
Vympel	25.67	328	189.28	0.84	0.48	8422.7	8232.6
Baikal	22.76	394.4	256	0.89	0.58	8976.6	8245.5
Humir	28.95	314.4	240	0.91	0.69	9100.6	8910.2
Humir-1	24.66	386.4	240	0.96	0.5919	9529.6	9108.6

Treatment of seeds with “Humir” preparations (with and without carotene) affected wheat productivity differently during the heading phase. Almost all indicators were higher than when using “Baikal” and “Vympel” as preparations of comparison (Table 5).

In the case of seed treatment with “Vympel”, a larger number of plants per 1 m^2 was noted than when treated with “Baikal”. Treatment only with hydrated fullerenes did not affect plant growth productivity positively. Treatment of plants with the "Humir-1" preparation, containing carotene, produced in a larger number of plants and spikelets, as well as an increase in the dry weight of plants compared to the preparation which did not contain carotene.

5. Spring wheat 2020, earing phase, mass of plants

Variant	Number per 1 m^2			Dry mass g/m^2, 1 hectare	Dry mass of 100 plants, g
	plants	spikelets	stem		
Control	248.9±23	234.4±	371.2±	348.8±	109.5±
Water with hydrated fullerene	237.2±	218.6±	298.6±	325.2±	107.0±
Vympel	266.4±	234.4±	310.4±	306.6±	115.1±
Baikal	244.4±	236.1±	364.3±	376.2±	137.0±
Humir	250.4±	240.6±	384.8±	370.9±	143.9±
Humir-1	259.0±	246.9±	386.9±	380.8±	150.9±

Unfortunately, because of weather conditions in 2019 (lack of rain), the wheat harvest was insignificant, but the seeds treated with growth-stimulating preparations gave a higher yield, the increase in yield was 10-11% higher than in the control (Table 6).

6. Accounting for the 2020 spring wheat harvest, average indicators

Variant	Weight of plant, centner /ha	Increase in yield, %
Control	11.36±12.0	---
Water with hydrated fullerene	12.47±13.3	9.83±0.9
Vympel	13.40±14.2	11.02±2.1
Vympel-2	12.39±13.4	9.13±1.02
Baikal	12.45±12.8	8.47±0.9
Humir	12.50±11.5	10.05±1.1
Humir-1	13.56±13.1	10.59±0.9

The influence patterns of preparations established in growing wheat were also observed in experiments on barley grown in rolls (Table 7).

7. Average indicators of the first series of experiments when growing barley in rolls

Variant	Germination energy, %	Germination ,%	Seeds,%		
			swollen	rotten	abnormally germinated
Control dry	74.5	77.5	3.0	7.5	7.5
Water	87.0	79.5	4.5	8.0	5.0
Water with hydrated fullerene	65.2	69.3	6.4	10.0	20.4
Vympel	85.0	86.0	5.0	5.5	5.5
Humir	87.3	86.0	3.9	5.9	2.9/100
Humir-1	85.0	88.5	7.5	6.0	6.0/100

Indicators of germination energy and germination exceeded control values. In addition, under the action of the preparation "Humir" a decrease in the number of abnormally germinated seeds was observed compared to "Humir-1".

8. Barley germination on the 10th day in rolls

Variant	Degree of germination								
	poor			medium			intensive		
	quantity		length, mm	quantity		length, mm	quantity		length, mm
	number	%		number	%		number	%	
Control dry	4	8.2	67.5	27	55.1	148.3	18	36.7	193
Water	3	6.5	74.4	23	50.0	149	20	43.5	196
Water with hydrated fullerene	2	4.8	68.2	19	45.2	146	21	50.0	200
Vympel	2	4.3	66.1	19	40.4	144	26	55.3	193
Humir	2	4.3	73.5	24	51	145	25	44.7	191
Humir-1	3	6.0	74.6	23	46	150	27	48.7	205/100

It should be noted that the preparation “Humir” had good wettability and a film-forming effect. After spraying, a thin film of the preparation which being absorbed by the plant for several days formed on the surface of the leaf. Due to this, “Humir” can be successfully used to combat diseases such as powdery mildew, late blight, bacteriosis, etc. If foci of disease occur on the leaves and stems of plants, careful treatment of the stems and leaves with the preparation ensures the fixation of fungal spores, blocking the spread of the disease.

Conclusions and perspectives

1. The laboratory experiments to evaluate the effectiveness of application of a new growth-stimulating preparation on seed germination have been carried out. As a result, it has been established that in the first stages of plant cultivation the preparation provided a stimulating effect, ensuring an increase in the length and weight of seedlings in the test variants compared to the control ones, respectively.

2. It has been established that the introduction of fullerene or fullerene-like substances (shungite) and microbiological carotene into the growth-stimulating preparation provided a positive effect on all indicators of the growth and development of grain crops (wheat, barley).

3. A decrease in the number of rotten and abnormally germinated seeds was observed, that may indirectly indicate the bactericidal effect of the preparation being developed.

4. The presence of hydrated fullerenes in solutions made it possible to develop a preparation that did not undergo separation during storage due to

the binding properties of fullerenes and provided bactericidal properties.

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ВИКОРИСТАННЯ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН У ПРЕПАРАТАХ ДЛЯ СІЛЬСЬКОГО ГОСПОДАРСТВА

Л. В. Кричковська, М. А. Бобро, С. А. Карпушина, Н. В. Хохленкова

Анотація. Актуальність. *Виробництво та застосування стимуляторів росту рослин, як у вигляді самостійних препаратів, так і в комплексі з традиційними органо-мінеральними добривами, має великий науковий інтерес і практичне значення. У зв'язку з цим одним із найбільш перспективних підходів до вирішення даної проблеми є розробка препаратів на основі регуляторів росту рослин, у тому числі з біостимуляторами, отриманими мікробіологічним синтезом і біостимуляторами, отриманими шляхом хімічного синтезу.*

Мета. *Розробити ростстимулюючий препарат з нетрадиційної сировини та випробувати його в лабораторних та польових умовах на дослідних партіях насіння ячменю та пшениці, що інкапсульовані досліджуваним препаратом, який містив наступні компоненти: гумат натрію, гідратовані фулерени, водний розчин шунгіту, каротин мікробіологічний у певному співвідношенні.*

Методи. *Лабораторно-польові досліди з вивчення впливу застосування препаратів з різними компонентами на ріст і розвиток ячменю та ярової пшениці проводили на базі Харківського національного аграрного університету імені В. Докучаєва на кафедрі рослинництва у 2020 р.*

Результати. *Результати, отримані під час лабораторних досліджень, показали, що застосування препарату «Гумір» мало стимулюючу дію на ріст рослин. Сходи в дослідному варіанті з'явилися на добу раніше контрольних, а рослини дослідного варіанту випереджали у розвитку контрольні. У підсумку*

середня довжина проростків у контролі становила 9,5 см, а в дослідному варіанті – 13,8 см. Середня маса проростків у дослідному варіанті становила 1,6 г. Таким чином, у попередніх лабораторних дослідженнях показано, що застосування препарату на перших етапах росту рослин мало стимулюючу дію, забезпечуючи збільшення довжини та маси проростків у дослідних варіантах порівняно з контрольними.

Висновки та перспективи. *Проведено дослідження та розробку нових нетрадиційних препаратів, які поряд із відомими стимуляторами росту рослин підвищують урожайність сільськогосподарських культур у харчовому виробництві. Розроблений препарат «Гумір», крім підвищення врожайності зернових культур, мав добру змочуваність та плівкоутворюючу дію. Після обприскування на поверхні листя утворювалася тонка плівка, яка протягом кількох днів поглиналася рослиною. Завдяки цьому «Гумір» також можна успішно застосовувати для боротьби з такими хворобами, як борошниста роса, фітофтороз, бактеріоз тощо.*

Ключові слова: *ростстимулятори, фулерени, шунгіт, гумати, каротин, біологічно активні речовини*