BIOTESTING AS A METHOD FOR ASSESSING THE STIMULATING EFFECT OF HUMIC COMPOUNDS ON HIGHER PLANTS

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Abstract. When improving agricultural technologies, considerable attention should be paid to the use of organic fertilizers, which involves the use of humic and fulvic acids. This will reduce the use of mineral fertilizers and increase the yield of agricultural products, as well as grow environmentally friendly products. Justification of the use of organic fertilizers to stimulate plant growth requires a preliminary study of their action under laboratory conditions. The article analyzes the wide application of biotesting procedures based on the reactions of living organisms, using plant test-objects. The availability of a wide range of plants allows their use for testing various factors, including the analysis of the stimulating effect of substances on higher plants. The purpose of the study was to study the effect of the organic fertilizer «Greenat» on the initial growth processes of higher plants in the laboratory by biotesting. The methodology of the study was to apply the biotesting method using higher plants widely used in agriculture, namely barley (*Hordeum vulgare* L.), soybeans (*Glycine max* L.), wheat (*Triticum aestivum* L.), maize (Zea mays L.), cucumber (*Cucumis sativus* L.), tomato (*Solanum lycopersicum* L.). It is established that the declared organic fertilizer «Greenat» contains: humic acids (67,68 g/dm^3), fulvic acids (24,37 g/dm^3) and organic substances (53,39%). The stimulating effect of the studied fertilizer on the initial processes of growth and formation of the root system of representatives of the group of cereals – barley (*Hordeum vulgare L.*) and wheat (*Triticum*

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aestivum L.) was revealed. Stimulating effect of organic fertilizer is also established for the initial growth processes of soybeans by estimating the length of shoots (increase by 38%) and for the initial growth processes of corn by estimating root length (increase by 22%). Root length of cucumber and tomato increased by 23 and 21% respectively, indicating the stimulating effect of the organic fertilizer «Greenat» in the treatment of seeds of vegetable crops. The results of the research indicate the effectiveness of using the organic fertilizer «Greenat» at the stage of seed treatment to stimulate the energy of germination and development of the root system of plants.

1. Introduction

The widespread use of chemicals has made great strides in the national economy, but their accumulation in the biosphere poses a real danger to all living beings. The problem of reducing contamination of the biosphere by searching for additional environmentally safe means of plant protection and increasing the nonspecific resistance of various organisms to the action of dangerous factors is becoming increasingly important.

Physiologically active humic compounds widespread in nature are promising in this direction. They are found in rivers and sea water, in soil, in peat and brown coal. These compounds are components of ecosystems and can modify the effect of biosphere pollutants on various organisms, preventing negative consequences [1].

In this regard, when improving agritechnologies, considerable attention should be paid to the use of organic fertilizers, in particular preparations with significant content of humic and fulvic acids. This will reduce the consumption of mineral fertilizers, reduce the effect of stress, stimulate the growth and development of plants, which will have a positive impact on the yield of agricultural products, as well as contribute to the cultivation of environmentally friendly products.

Modern studies show that humic acid molecules are characterized by unique properties due to their organic origin and structural features. They are also used in animal husbandry and even in medicine [1].

Fulvic acids are biologically active compounds soluble in water, acids and alkalis. The size of fulvic acids is small, which ensures high mobility of these substances. They translate nutrients into a form accessible to the plant due to their chelating. Humic acids are biologically active substances of a complex structure, almost insoluble in water, except for a very small part of them. Treatment of humic acids with alkalis turns them into water-soluble salts – sodium or potassium humates, which exhibit biological activity. Humic acids promote the development of a strong root system, prevent stresses of various nature, activate antioxidant enzymatic function, increase the absorption of such important nutrients as nitrogen (2 times), phosphorus (1.8 times) and potassium (3-4 times).

Humates and preparations based on them significantly differ from each other by the method and technological conditions of production, raw materials, form of production, biological activity, solubility, conditions of application. This necessitates preliminary (prior to application) study of their action in laboratory conditions and preference is given to humates with guaranteed results.

The widespread use of bioassay procedures using plant test objects to determine the toxicity of chemical compounds suggests that they can be used to evaluate both the phytotoxic and stimulatory effects of substances on higher plants.

The work is to study the effect of the organic fertilizer "Greenat" on the initial growth processes of higher plants in the laboratory by biotesting.

2. Using the biotesting method on plant test objects

Current trends in ecological control of exogenous influences increasingly reflect a biotic approach. Chemical analysis indicates only the presence of "markers" – some concentrations of pollutants, when compared with standard concentrations, and does not allow to assess the state and prospects of development of various components of the biota and the ecosystem as a whole in the case of pollution [2–4]. First of all, it concerns obtaining objective information about the secondary effects of pollutants due to their accumulation and transformation by various parts of ecosystems, the holistic nature of the response to external influences [5].

Biotesting is testing based on the reaction of living organisms to the action of factors under experimental laboratory or field conditions. They consist of recording changes in biologically relevant indicators of test systems, followed by evaluation according to selected criteria. Active biotesting is performed under strictly defined conditions for living organisms in comparison with the control. Field studies of visible or latent lesions of organisms on the background of the control, which are signs of adverse effects of ecotoxicants, are passive biotesting [3].

Test-objects (test-organisms) are biological objects (organisms) used in assessing the toxicity of chemicals. The use of test objects in many cases makes it possible to replace complex chemical analyses. Organisms used in biotesting are cultured under standard conditions. With this approach, the choice of the test object and the parameters for changing its life activity is essential. Sensitive test objects include soil and water microorganisms, protozoa, crustaceans, mollusks, most insects, worms, etc. [5]. The possibility of using model plant objects in the conditions of growing plants in aquatic culture has been shown to assess the effect of exogenous impact on biota [6].

Plants are convenient objects for biotesting, bioindication or biomonitoring. Indicator plants are plants that show signs of change (damage) when exposed to phytotoxic concentrations of substances. Representatives of natural flora are used as phytoindicators, and one of the selection criteria is their distribution. Plant seedlings are convenient objects of research [7].

Plant populations, correctly reflecting the state of the ecosystem, are reliable test-systems for assessing endogenous action [8]. The overall response of plants to the complex impact of anthropogenic factors is assessed by indicators that have biological meaning. Phytocenotic studies take into account the floristic composition of the respective areas, the number of individuals per unit area, their biomass, population structure, viability of individuals, etc. [9].

Plant test objects are plant organisms used in in situ biotesting experiments under laboratory and natural conditions [10]. Biotesting can also be used to determine the stimulatory effects of substances on plants. Experiments in which toxicity or stimulation is determined are called bioassays (biotesting).

The specific purpose of the study is of great importance for the choice of test objects. All test systems have their own sensitivity range and allow detecting a specific type of exposure. There is no universal test-system that simultaneously registers the entire range of lesions. Under deterministic conditions, it is recommended to use test-objects whose response is based on nonspecific reactions.

The availability of a wide range of plants allows their use for testing various factors of chemical and physical nature [6]. Tests using wild and agricultural plants are useful.

One of the types of biotesting is the growth test, which takes into account the change in the germination indices of the test plant grown on the studied samples of soil, water, aqueous soil extracts, etc. This method allows evaluating not only the inhibitory effect of various pollutants on plants, but also the stimulating one. The most common test plants are wheat, cucumber and lettuce. Variants of growth test: 1) germination of test plants in Petri dishes; 2) germination of test plants on floating disks; 3) ermination of test plants on substrate.

Plants are kept under experimental conditions and the following indicators are recorded:

– the time of emergence of seedlings and their number (every day);

– the length of the aerial part of the seedlings and their growth (every day);

– total number of germinated seeds (at the end of the experiment).

Attention is paid to the morphological features of plants (early yellowing, peculiarities of root system development, etc.).

After two weeks, the young plants are carefully released from the water and dried a little on filter paper. Then the length of the root and stem system is measured. The plants are then placed in paper bags, dried for several days, and then the dry weight is determined. The absence of statistically significant difference between the average values of a particular biological parameter (plant weight, length of root or stem systems, the number of damaged plants or the number of seedlings, etc.) in the control and the studied variant indicates the absence of significant changes. A statistically significant difference between the variant and the control experiment indicates a phytotoxic effect (decrease in the value of the index) or a stimulating effect (increase in the value of the index) [11].

Bioassay methods are quick to perform; accessible and simple; highly reproducible and reliable; cost-effective both in terms of material and labor costs; objective in terms of the results obtained; make it possible to assess the combined effect of factors, including in conditions of chronic (weak) action [12–14].

Consider the advantages of using a test system based on higher plants as compared to other test systems for the assessment of mutagenic active factors. Plants are eukaryotes, and the results obtained can be extrapolated to other eukaryotic organisms, including humans. These tests are relatively inexpensive, short-lived, easy to use, and highly sensitive. The methods of some of them are well developed and standardized. These tests do not require sophisticated laboratory equipment. Some of the plant testing systems have short life cycles. The tests can be used under various environmental conditions, pH, temperature. Higher plants can be regenerated from a single galloid or diploid cell. Both single substances and complexes of mixtures can be used for testing. Higher plants are highly sensitive to the detection of carcinogenic agents. Genotoxicity of some compounds can be determined under the same conditions in several test systems at once, which allows the comparison of results. There are some limitations in the use of test systems of higher plants to determine genotoxicity, but tests on plants are actively used to determine various mutagenic factors, as well as for biotesting of environmental pollution [15].

Biotesting by technology is an experiment carried out in accordance with certain methodological requirements. However, research results often have a high variability (up to 35%) when reproduced in different laboratories [16]. The large intraspecific diversity of living organisms becomes a significant problem when conducting biotesting. There are various ways to minimize the scatter of test results and increase reliability and reproducibility. First of all, the test subjects of the same experiment should be visually identical. For example, seeds are calibrated by weight and size; for animals, age, sex, and weight are important. When studying the toxicity of substances on microalgae, an algologically pure 3-4-day-old culture is used and maintained under optimal conditions. Standardization is carried out for all external conditions such as light, temperature, humidity. The number of parallel experiments in any variant should be sufficient for further statistical processing.

A living organism reacts to external influences by a change in homeostasis, which can characterize the state of stress and influence the prognosis of further organism viability. The vast majority of biotesting methods use integral parameters of organisms reflecting their state as a whole: survival rate, reproductive function, growth rate, etc.

The test functions used in the various biotesting options are quite varied.

In toxicology, the main criterion of toxicity is the lethality or survival of cells and organisms. It can characterize the decline of a natural population or a laboratory culture (organisms) under the influence of a particular factor. Specifically, when using a cell culture, the relative content of live and dead cells can be estimated using special dyes or fluorescent microscopy.

Under exogenous influence it is possible to evaluate the dynamics of seed germination and the number of germinated seeds according to the following indicators. Percent germination is the percentage of seeds that when sown under favorable conditions (temperature, humidity, light) form normal seedlings (laboratory germination); viability is the percentage of seeds that form normal seedlings under field or greenhouse conditions (field germination); germination energy is the percentage of seeds that can germinate within a short time from the start of the germination test in the laboratory (expressed as the average number of days needed for one seed to germinate). Not whole plants can be used for biotesting, but their individual parts and isolated organs.

In plants (algae, higher plants), the most indicative reaction to toxic effects is a decrease in the intensity or complete cessation of photosynthesis; in addition, the content of chlorophylls and other plant pigments is determined.

Signs of chronic plant damage include bronzing of leaves, chlorosis and discoloration, and premature ageing.

An imbalance between antioxidant activity and lipid peroxidation can be observed in plants under stressors. In this case, LPO and antioxidant activity parameters can act as biomarkers for assessing the degree of exogenous exposure.

The response of plant test systems to exogenous influences is evaluated by physiological (growth rate), histological, cytological (mitotic index, chromosomal aberrations, micronucleus test, etc.) or other parameters.

The introduction of new biotesting methods is monitored by international organizations such as the European Center for the Validation of Alternative Methods (ECVAM), the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM).

Given the widespread use of biotesting and bioindication methods based on the use of plant test systems, the problem of their standardization remains relevant. The International Organization for Standardization (ISO), in particular, is engaged in standardization of biotesting methods, including the use of plant test objects [18–19].

3. Study the effect of organic fertilizers on the initial growth processes of higher plants

The objects of research were plants-biotests: barley (*Hordeum vulgare* L.), soybean (*Glycine max* L.), wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), cucumber (*Cucumis sativus* L.), tomato (*Solanum lycopersicum* L.). Seeds of the above model crops with high sowing qualities (germinating capacity ≥ 90%) according to [18] were used in the experiments.

The study was conducted according to [18–19]. Plants selected for testing according to the recommendations belonged to two classes: monocotyledonous (barley, wheat, corn) and dicotyledonous (soybean, common cucumber, common tomato). Control of convergence and in-laboratory reproducibility was performed according to [19].

Research methods

Seeds were soaked for 10 hours in an aqueous solution of «Greenat» organic fertilizer in a concentration equivalent to the maximum recommended by the customer dose (20 ml per 10 L of water) to stimulate the transition from dormancy to enhanced growth and development. Seeds soaked in stagnant tap water served as a control. Sterile Petri dishes were used to germinate the seeds.

The dishes with seeds were covered and germinated in the thermostat at 24 ± 1 °C for 48 h (until germination in the control of 90-100% of the total number of germinated seeds), after which the germinated seeds were counted. A germinated seed is a seed in which the root broke through the seed coat.

Then, within 5-7 days (depending on the plant species) the growth of seedlings and root system were recorded, and after the end of the experiment measurements were carried out. The experiment was conducted in 4-fold replication.

Phytotoxic/stimulating effect of substrates was determined by morphometric parameters (root and shoot length) of plants [15]. The length of the longest roots of each plant was measured and the average length of the shoot and the longest root for each experiment variant was determined. The results of the experimental variants were compared with the results in the control. In addition, the total weight of the root system was determined. For this purpose, the roots of plants from each experiment were dried and the dry weight was determined.

Mathematical processing

Mathematical processing of the results of the study was performed using the method of mathematical statistics; the difference was evaluated using Student's t-test according to [19]. Statistical processing of the data was performed using the SPSS Statistics 17.0 program. After measurements for the study variants, the average length of the above-ground and root parts was calculated $\bar{x} \pm m$, where m is the error of the arithmetic mean, determined by the formula:

$$m = \sqrt{\frac{\sigma^2}{N}}$$

Where N – number of results; $\tilde{A}^2$ – variance determined by the equation:

$$\sigma^2 = \frac{\sum_{i=1}^{N} (x - \bar{x})^2}{N}$$

The reliability of the arithmetic mean t difference is calculated using Student's t-test:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{m_1^2 + m_2^2}}$$

Where $\bar{x}_1$ – the arithmetic mean of the indicator in the control version;

$\bar{x}_2$ – the arithmetic mean value of the indicator in the studied variant;

m_1 – arithmetic mean error in the control version;

m_2 – arithmetic mean error in the studied version.

If the actual t value is greater than or equal to the critical (standard) value of t_{st}, then the conclusion is made that there is a statistically significant difference between the arithmetic mean of the studied and control variants.

Characteristics of the organic fertilizer «Greenat»

The product «Greenat» (declared as an organic fertilizer), which is presented in the preparative form – black liquid substance, was tested. According to the studied parameters (Table 1), the product is enriched with humic and fulvic acids, which content was determined according to [20–21]. The content of organic substances, according to [22], is 53,4%. Thus, the product can be characterized as an organic fertilizer.

Table 1

Physico-chemical parameters of the organic fertilizer «Greenat»

Indicators	Measurement results
Salt extract pH, pH units	7,32±0,02
Weight by volume, g/cm^3	1,05±0,12
Humic acid concentration, g/dm^3	67,68±4,06
Free humic acid concentration, g/dm^3	56,07±3,46
Fulvic acid concentration, g/dm^3	24,37±1,71
Organic substances (carbon), %	53,39±1,32
Mass fraction of the total amount of potassium (K_2O), %	0,09±0,01

Note: The results are presented on the natural state of the sample

Experimental research

Under laboratory conditions with barley (*Hordeum vulgare L.*) it was found that the use of organic fertilizer "Greenat" in a concentration equivalent to the recommended maximum rate of application, increases the number of germinated barley seeds compared with control. When using the organic fertilizer "Greenat" the length of sprouts and roots increased by

Table 2

The effect of the organic fertilizer «Greenat» on seed germination and growth processes of barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), maize (*Zea mays* L.)

Experiment options	Indicators			
	Seeds germination, %	Sprouts length, mm	Roots length, mm	Total mass of roots**, g
Barley (*Hordeum vulgare* L.)				
Control	91	13,48±1,08	27,41±0,68	0,097±0,004
Organic fertilizer «Greenat»	97	16,40±1,39*	32,22±0,86*	0,145±0,011*
Wheat (*Triticum aestivum* L.)				
Control	93	29,47±1,83	57,15±1,26	0,110±0,005
Organic fertilizer «Greenat»	95	32,25±1,70	65,32±1,71*	0,139±0,010*
Maize (*Zea mays* L.)				
Control	95	43,3±1,5	52,47±0,97	-
Organic fertilizer «Greenat»	98	44,8±1,1	63,79±1,16*	-

Note: * − P< 0,01 regarding control, ** − calculation for dry weight.

22 and 18%, respectively, relative to the control variant. In addition, under experimental conditions there is an activation of branching roots formation. The total mass of the roots increases by 49% (Table 2).

That is, the development of the root system is improved. Thus, the use of the organic fertilizer "Greenat" has a stimulating effect on seed germination and initial growth processes of barley (Figure 1).

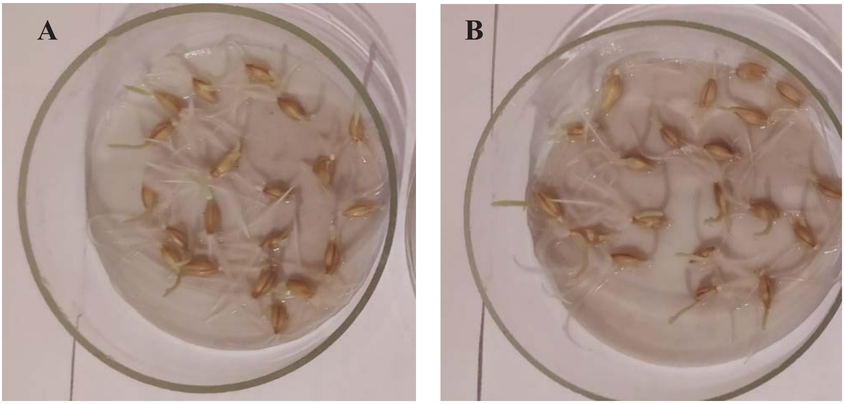

Figure 1. The effect of the organic fertilizer «Greenat» on initial growth processes of barley (*Hordeum vulgare* L.): A − control, B − application of organic fertilizer «Greenat»

Under laboratory conditions with wheat (*Triticum aestivum L.*) it was found that the use of organic fertilizer «Greenat» in an amount equivalent to the recommended maximum rate of application, the length of shoots and roots increased by 9 and 14%, respectively, relative to the control variant. In addition, under experimental conditions, the activation of branching roots formation was established. The total mass of roots increases by 26% (Table 2). That is, the development of the root system is improved. The total number of germinated wheat seeds practically does not differ from the control. Thus, the use of organic fertilizer «Greenat» has a stimulating effect on the initial growth processes of the root system of wheat (Figure 2).

Maize, as a crop of long growing season, is able to absorb nutrients from the soil throughout its life cycle. Under laboratory conditions, it was found

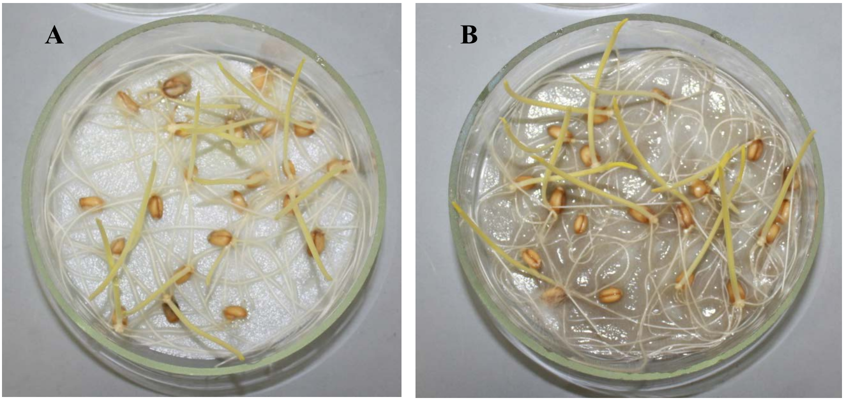

Figure 2. The effect of the organic fertilizer «Greenat» on the initial growth processes of wheat (*Triticum aestivum* L.): A – control, B – application of organic fertilizer «Greenat»

that when using organic fertilizer "Greenat" in an amount equivalent to the recommended maximum rate of application, the root length increased by 22% relative to the control variant (Table 2, Figure 3). At the same time, it does not affect the length of sprouts and the total number of germinated

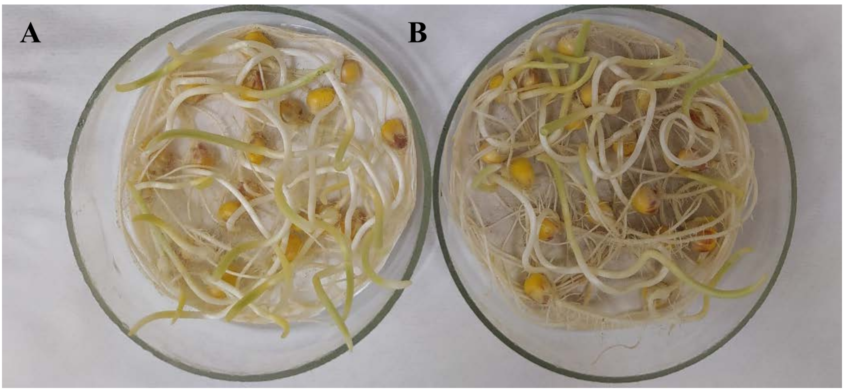

Figure 3. The effect of the organic fertilizer «Greenat» on the germination of seeds and the initial growth processes of maize (*Zea mays* L.): A – control, B – application of organic fertilizer «Greenat»

seeds. Thus, the use of organic fertilizer «Greenat» has a positive effect on the process of initial root formation in maize.

Soybean (*Glycine max* L.) is a crop that responds positively to fertilizers, which contributes to obtaining high and stable yields of its grain. Under laboratory conditions it was found that when using the organic fertilizer «Greenat» in an amount equivalent to the recommended maximum rate of application, the length of soybean seedlings increased by 38% relative to the control variant (Table 3, Figure 4). The total number of germinated seeds does not differ from the control.

Table 3

The effect of the organic fertilizer «Greenat» on the number of germinated seeds and the length of soybean sprouts *(Glycine max L*.), roots length of cucumber (*Cucumis sativus* L.) and tomato (*Solanum licopersicum* L.)

Experiment options	Indicators	
	Seeds germination, %	Sprouts length or Roots length, mm
Soybean (*Glycine max* L.)		
Control	95	49,20±0,73
Organic fertilizer «Greenat»	94	67,97±0,89*
Cucumber (*Cucumis sativus* L.)		
Control	93,3	44,71±0,80
Organic fertilizer «Greenat»	95,6	55±0,85*
Tomato (*Solanum licopersicum* L.)		
Control	91	16,25±0,46
Organic fertilizer «Greenat»	95	19,67±0,55*

Note: * − P< 0,01 regarding control.

Therefore, the use of organic fertilizer “Greenat” has a stimulating effect on the initial growth processes of soybeans.

Under laboratory conditions, when applying the seeds of cucumber, it was found that using the organic fertilizer “Greenat” in an amount equivalent to the recommended maximum rate of application, cucumber root length increases by 23% relative to the control variant (Table 3, Figure 5).

The total number of germinated seeds does not differ from the control. Thus, the use of organic fertilizer “Greenat” has a stimulating effect on the initial growth processes of cucumbers.

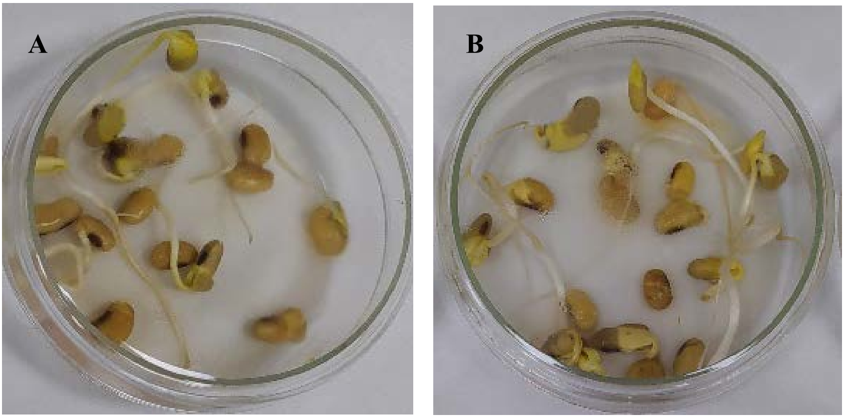

Figure 4. The effect of the organic fertilizer «Greenat» on the initial growth processes of soybean (*Glycine max* L.): A − control, B − application of organic fertilizer «Greenat»

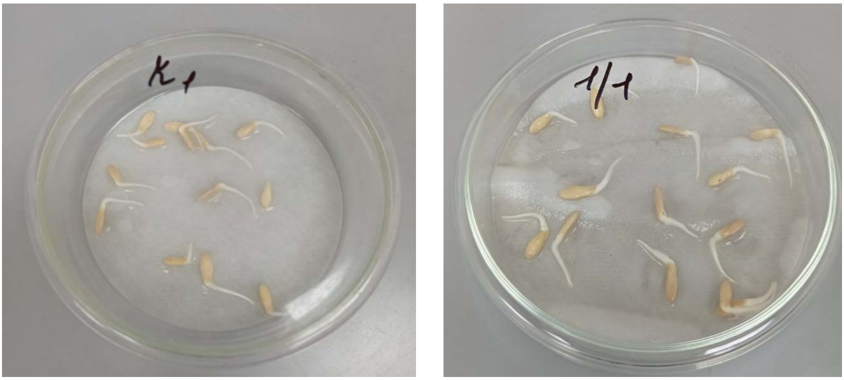

Figure 5. The effect of the organic fertilizer «Greenat» on the initial growth processes of cucumber (*Cucumis sativus* L.): $К_1$ – control, 1/1 – application of organic fertilizer «Greenat»

Under laboratory conditions with tomato (*Solanum lycopersicum* L.) it was found that when using the organic fertilizer "Greenat" in an amount equivalent to the recommended maximum rate of application, the root length of tomato seedlings increased by 21% relative to the control variant (Table 4). The total number of germinated seeds practically does not differ from the control. Consequently, the use of the organic fertilizer "Greenat" has a stimulating effect on the initial growth processes of tomatoes (Figure 6).

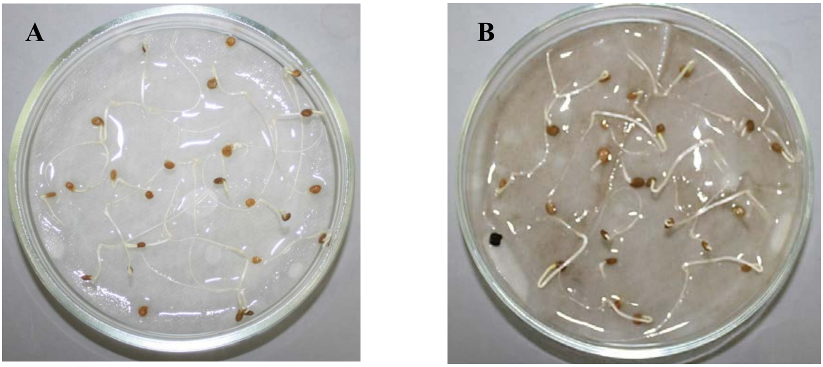

Figure 6. The effect of the organic fertilizer «Greenat» on initial growth processes of common tomato (*Solanum lycopersicum* L.): A − control, B − application of organic fertilizer «Greenat»

Under laboratory conditions, it was found that during the initial stages of plant growth, plant roots grow the most (compared with the control). When growing soybean, the growth of seedlings is observed (compared with the control). The arithmetic mean value of root (sprout) length and variance were calculated according to the above formulas. The results are presented in Table 4. Then, the reliability of the obtained results was determined in comparison with the control. It was found that for each variant of the experiment t value > t_{st} (0,01) = 2,63, therefore, the obtained results are significantly different from the control.

Thus, under laboratory conditions, phytotoxic effect of organic fertilizer "Greenat" (in the rate recommended by the customer) (20 ml/10 l of water) with respect to germination and initial growth of higher plants was not detected: barley (*Hordeum vulgare* L.), soybean (*Glycine max* L.), wheat (*Triticum aestivum* L.), maize *(Zea mays* L.), cucumber (*Cucumis sativus* L.), tomato (*Solanum lycopersicum* L.). It was found that the use of organic fertilizer "Greenat" has a positive effect on seed germination.

Table 4

Arithmetic mean values of plant root (seedling) length, error and variance for each variant

Plants	Experiment options	Variance σ^2	Average $\bar{x}$±m	t-test
Barley *Hordeum vulgare* L. (for roots)	Control	5,04	27,41±0,68	-
	Organic fertilizer «Greenat»	6,57	32,22±0,86	4,39
Wheat *Triticum aestivum* L. (for roots)	Control	9,51	57,15±1,26	-
	Organic fertilizer «Greenat»	12,94	65,32±1,71	3,86
Soybean *Glycine max* L. (for shoots)	Control	5,59	49,21±0,73	-
	Organic fertilizer «Greenat»	6,76	67,98±0,89	16,31
Maize *Zea mays* L. (for roots)	Control	7,38	52,47±0,97	-
	Organic fertilizer «Greenat»	8,80	63,79±1,16	7,48
Cucumber *Cucumis sativus* L. (for roots)	Control	5,20	44,71±0,80	-
	Organic fertilizer «Greenat»	5,50	54,96±0,85	8,78
Tomato *Solanum lycopersicum* L. (for roots)	Control	3,52	16,25±0,46	-
	Organic fertilizer «Greenat»	4,17	19,67±0,55	4,76

Note: t value > t_{st} (0,01) = 2,63.

Under experimental conditions, under morphometric parameters in plants the stimulating effect of initial growth processes (mainly the root system) is observed. In addition, for wheat and barley, along with an increase in root length, stimulation of root system tillering is observed. This indicates that the roots of plants with access to humic acids grow fibrous and branched. These acids are excellent plant growth stimulators. And as it is known, a well-developed root system provides the above-ground part of plants with enough nutrients.

In the recommendations for the use of humates, part of the total recommended amount of this product is used for seed and material treatment, part is separated out for foliage application, and the rest is used to feed the root system of plants. Under laboratory conditions was shown high efficiency of seed treatment with an organic fertilizer (growth promoter)

"Greenat" to increase the germination energy and stimulate the formation of the root system of crops with active specific weight.

4. Conclusions

The results of studying the effect of organic fertilizer "Greenat" in the recommended rate (20 ml/10 l of water) as an organic fertilizer (growth promoter) on the germination and initial growth of higher plants in the laboratory conditions indicate the following:

1. The claimed organic fertilizer "Greenat" contains: humic acids (67,68 g/dm^3), fulvic acids (24,37 g/dm^3), organic substances (53,3 %), potassium, K_2O (0,1%).

2. The stimulating effect of the studied fertilizer on the initial processes of growth and formation of the root system of cereals – barley (*Hordeum vulgare L.*) and wheat (*Triticum aestivum L.*) was revealed.

3. According to the indicator of root length (increase by 22%) the stimulating effect of organic fertilizer on the growth processes of maize (*Zea mays L.*) roots was established.

4. There was a stimulating effect of organic fertilizer on the initial growth processes of soybeans (*Glycine max L.*) on the length of sprouts (increased by 38%).

5. Root length of cucumber (*Cucumis sativus L.*) and tomato (*Solanum lycopersicum L.*) increased by 23 and 21% respectively, indicating the stimulating effect of the organic fertilizer «Greenat» in the treatment of vegetable seeds.

6. The results indicate the effectiveness of the organic fertilizer "Greenat" at the stage of seed treatment to stimulate germination energy and development of the root system of plants.

References:

1. Horovaya A. I., Orlov D. C., Scherbenko O. V. (1995). Ghumynovue veschestva. Stroenye, funkcyy mekhanyzm dejstvyja, protektornue svojstva. Ekologhycheskaya rol [Humic substances. Structure, function, mechanism of action, protective properties. Ecological role]. Kyiv: Naukova dumka. (in Ukrainian)

2. Biotestuvannia yak metod otsinky yakosti pytnykh vod (2006) [Biotesting as a method of assessing drinking water quality]. *Visnyk NAN Ukraine*, 10, 54–57.

3. Liashenko O. A. (2012). Bioindikatciya u biotestirovanie v ohrane okryzhajushchey sredu [Bioindication and biotesting in environmental protection]. St. Petersburg: HTYRP. (in Russian)

4. Isidorov V. A. (1999). Vvedenie v khimicheskyyu ekotoksikologiyu [Introduction to Chemical Ecotoxicology]. St. Petersburg: Khimisdat, pp. 127–129. (in Russian)

5. Zhirnov V. V., Savchenko D. A. (2017). Biokonversiya vidkhodiv [Waste bioconversion]. Ch. I. Kyiv: DDP «Ekspo-Dryk». (in Ukrainian)

6. Grodzinskiy D. M., Mikheev O. M., Kravets O. A. et al. (2003). Henotoksychnyi vplyv radionuklidnoho zabrudnennia hruntiv na merystemy roslyn [Genotoxic effect of radionuclide soil contamination on plant meristems] / *Zbirka naykovikh prats Instytutu yadernykh doslidzhen*, 2(10), 61–71.

7. Gorova A. I., Skvortsova T. V., Klimkin I. I. et al. (2005). Tsytohenetychnyi monitorynh dovkillia ta zdorovia liudyny [Cytogenetic monitoring of the environment and human health]. *Visn. Ukr. tov-va henetykiv ta selektsioneriv,* 3(1–2), 36–47.

8. Klimenko M. O., Prishchepa A. M., Voznyuk N. M. (2006). Monitorynh dovkillia [Environmental monitoring]. Kyiv: Akademiia. (in Ukrainian)

9. Glykhov A. Z., Kharkhota A. I., Nazarenko A. S., Likhanov A. F. (2005). Teratohenez rastenyi na yuho-vostoke Ukrayni [Teratogenesis of plants in the south-east of Ukraine]. Donetsk: NORD-PRESS. (in Ukrainian)

10. Arutyunyan R. M., Pogosyan V. S., Simonyan E. H. (1999). In situ monitoring of the ambient air around the chloroprene rubber industrial plant using the Tradescantia-stamen-hair mutation assay. *Mutation Research*, 426, 117–120.

11. Khyzhnyak S. V. Baranov Yu. S., Demchenko V. F., Voitsytskiy V. M. (2019). Pestytsydy ta yikh ekoloho-toksykolohichna otsinka [Pesticides and their ecological and toxicological evaluation: monograph]. Kyiv: NULES Ukraine. (in Ukrainian)

12. Smith J. L., Paul E. A., Bollag J., Stotzky M. (1988). The significance of soil microbial biomass estimations. *In Soil biochemistry*, 6, 357–396.

13. Khyzhnyak S., Nezbrytska I., Velynska A., Kalachniuk L. (2021). Toxicity Assessment of Individual and Combined Pesticides for Biota / Research Trends in Agriculture Sciences. V. 26 / AkiNik Publications. New Delhi. India, pp. 133–157.

14. Trevors J. T. (1984). Effect of substrate concentration, temperature and pH on dehydrogenase activity in soil. *Plant Soil*, 77, 285–293.

15. Grodzinsky D. M., Shylina Yu. V., Kutsokon N. K. et al. (2006). Zastosuvannia roslynnykh test-system dlia otsinky kombinovanoi dii faktoriv riznoi pryrody [Application of plant test systems to assess the combined action of factors of different nature]. Kyiv: Phytosocial Center. (in Ukrainian)

16. Kyrilenko V. V. (2004). Osnovi ekoheolohiy, byoyndykatsiy y biotestyrovanyia vodnikh ekosystem [Fundamentals of ecogeology, bioindication and biotesting of aquatic ecosystems: tutorial / edited by V. V. Kyrilenko. St. Petersburg. (in Russian)

17. Melekhova O. P. (2007). Byolohycheskyi kontrol okruzhaiushchei sredы: byoyndykatsyia y byotestyrovanye [Biological control of the environment: bioindication and biotesting: tutorial / edited by O. P. Melekhova, E. I. Egorova. Moscow: Akademiia. (in Russian)

18. ISO 11269–1:2002. Soil quality. Determination of the effects of pollutants on soil flora − Part 2: Effects of chemicals on the emergence and growth of higher plants.

19. ISO 11269–1:2002. Soil quality. Determination of the effects of pollutants on soil flora − Part 1: Method for the measurement of inhibition of root growth.

20. DSTU 7083:2009 Manure and organic-minerals fertilizers. Methods of humus and fulvic acids determination.

21. DSTU 7855:2015 Soil quality. Determination of group gumus composition on method of Tjurin in Kononova and Belchikova's updating.

22. DSTU 8454:2015 Organic fertilizers. Organic determination methods.