Utilization of wastewater pollution in plant growth biostimulants

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Abstract. The Voronezh Region is a highly developed agro-industrial complex that exerts a burden on the environment. The article deals with the disposal of wastewater pollution with the production of technological products. The aim of the study was to create a biostimulator of plant growth from the waste water of a meat processing plant by enzymatic hydrolysis with the addition of 5% of a consortium of microorganisms from the intestines of pigs and to study its properties on the seeds of Scots pine. The resulting hydrolysates have a high biological value with a complete amino acid composition. The biostimulator contains 92.52% amino acids. Identification of growth stimulation of seed sowing qualities was determined in the laboratory. The results of the experiment show that in the control sample, the seeds were soaked with distilled water, the germination energy was 71.3%, and the laboratory germination rate was 82%. When studying the prototype impregnated with a biostimulator, the germination indicators were: germination energy 85.3%, germination 93.3%. The resulting amino acid biostimulator increased the shelf life of seeds by 11.3%. The biostimulator helped to increase the quality class of seeds, which allows to reduce the seeding rate per unit area during sowing and reduce costs.

1. Introduction

Modern agricultural production is impossible to imagine without the use of plant growth regulators. Their use activates the immune systems of plants, increases stress resistance. Today, the market for such drugs is huge. Many plant growth stimulators of chemical and biological origin have been created [1]. But in the transition to ecological agriculture, preference is given to the latter. As a rule, biostimulants are obtained from substances of natural origin – fungi, bacteria, algae, peat, coal, sapropel, etc. [2]. This group of substances also includes amino acids. Active research of the action of amino acids on plants began in the 70-80-ies of the last century. So the first biostimulator of animal origin based on amino acids and peptides Siapton ® was developed in Italy in 1969 from waste from slaughterhouses obtained by hydrolysis of raw materials to amino acids and peptides [3-5]. Siapton ® promoted the formation of roots, improved the absorption and transport of macro-and microelements by plants.

The analysis of the market of biostimulants showed a tendency of its increase. According to forecasts, by 2025, their production will reach 3,800,000,000 USD. In the Russian market, these products are mainly represented by preparations of foreign production. The development of domestic competitive biostimulants will solve the problem of import substitution.
Sources of proteinogenic amino acids can be various organic waste products. Most (up to 90%) of the amino acid biostimulants of plant growth are obtained by chemical hydrolysis of proteins [6,7]. While fermented hydrolysates allow you to preserve the entire complex of amino acids that make up the waste. In this regard, it is worth paying attention to the possibility of obtaining new non-traditional amino acid biostimulants from the waste water of meat processing plants by microbial fermentation with the microbiome of the gastrointestinal tract of slaughtered animals, the contents of which are washed into the sewer. Wastewater from meat processing plants contains up to 40% protein [8].

Microbial fermentation of wastewater pollution will create useful products of microbiological processing of organic waste, reducing the burden on the environment.

The aim of the study was to conduct a comparative assessment of the effect of amino acid biostimulants obtained by chemical and microbiological methods on the growth and development of plants.

2. Materials and methods

The objects of the study were wastewater from enterprises of the agro-industrial complex of Voronezh, the intestinal microbiome of pigs (LLC “Bobrovsky Meat Processing Plant”), seeds of scots pine collected at the end of November 2020.

Wastewater was subjected to enzymatic hydrolysis for 7 days at a temperature of 40±2°C. A consortium of microorganisms from the gastrointestinal tract of pigs was introduced into the wastewater at a concentration of 5% by volume of the runoff. As a control, wastewater was used without the addition of a consortium of microorganisms.

The total protein was determined on an automatic Turbotherm unit (Gerhardt), amine nitrogen-spectrophotometrically using 2,4,6-trinitrobenzenesulfonic acid. The degree of hydrolysis was determined as the ratio of amine nitrogen to total protein. The amino acid composition was determined by ion exchange chromatography with post-column derivatization with ninhydrin.

The study of the effectiveness of the biostimulator was carried out on the seeds of Scots pine (Pinus Sylvestris). The seeds were collected in the second half of November in a natural stand in the Voronezh region, which belongs to the V zone of seed harvesting.

The effect of stimulating the growth of seed sowing qualities (germination energy and germination rate) was determined in the laboratory, in accordance with the current GOST 13056.6-97 “Seeds of trees and shrubs. Method for determining germination” [9].

For germination, externally whole seeds were selected, soaked for 24 hours according to the scheme: control (in water), experiment (in a biostimulator), the ratio of the volume of seeds and solution 1:5. All studies were carried out in three-fold repetition. The prepared seeds were washed and placed in Petri dishes on a wet bed of filter paper folded in four layers. Petri dishes were placed in the “Sanyo MIR-154” thermostat. During seed germination, the bed was kept moist by periodically wetting the filter paper with distilled water. The temperature in the thermostat is 25-27 °C. Seed sprouts were counted on the 5th, 10th, 12th days, and germination energy was counted on the 10th. On the day of accounting, the number of sprouted and non-sprouted seeds was calculated. On the day of the final germination count, the number of seeds remaining on the bed was determined by the number of non-sprouted, rotted, steamed, empty and infected with pests. The obtained data was entered in the table.

3. Results

Many scientists note that amino acids exhibit immunomodulatory, anti-stress, regulatory properties [10], increase the ability to assimilate nutrients and resistance to pests and diseases [11], etc. In the works of other researchers, it is shown that in free form, amino acids are involved in the mechanisms of plant resistance and adaptation to stress, in the regulation of water and nitrogen metabolism, are precursors of phytohormones and other biologically active substances, have antioxidant properties, etc. (table 1) [11,12]. It should be noted that only proteinogenic (protein) amino acids are actively involved in metabolism, synthesized inside plants, and are well absorbed by them.
### Table 1. The role of amino acids in plants.

| Name of the amino acid | Functions |
|------------------------|-----------|
| Aspartic acid + asparagine | activates seed germination, a source of organic nitrogen |
| Threonine | regulates the operation of leaf stomata under unfavorable climatic conditions |
| Serin | promotes plant resistance in drought conditions |
| Glutamic acid + glutamine | stimulates seed germination, accelerates growth, participates in the synthesis of chlorophyll |
| Glycine | promotes the growth of tissues, the synthesis of chlorophyll and vitamins |
| Alanine | optimizes water exchange, increases plant resistance in drought conditions |
| Valine | activates the germination of seeds, improves the taste of fruits, increases the resistance of plants to adverse natural factors |
| Methionine | activator of phytohormones and substances that affect the growth and development of plants |
| Isoleucine | accelerates pollen germination, increases plant resistance in drought conditions |
| Leucine | increases plant resistance to heat, drought, and salinity |
| Tyrosine | a compound of proteins that increases the resistance of plants to heat, drought and salinity |
| Phenylalanine | affect the thickness of the cell walls, stimulates seed germination |
| Histidine | improves the absorption of nutrients |
| Lysine | participates in the synthesis of chlorophyll, increases resistance to drought, regulates the work of leaf stomata, activates pollen germination |
| Arginine | participates in the synthesis of hormones associated with the formation of flowers and fruits, promotes the penetration of nutrients into the root system |
| Proline | synthesizes chlorophyll, promotes moisture retention and gas exchange |
| Tryptophan | promotes the formation of the root system, helps the plant to overcome a stressful situation |

Non-proteinogenic amino acids can act as inhibitors, delaying certain steps of amino acid biosynthesis or contributing to the formation of false sequences in the synthesis of proteins [13,14].

According to the data presented in figure 1, the hydrolysis of organic waste by the gut microbiome of animals is more intense than in the control sample. After 24 hours of fermentation, the degree of hydrolysis was 16% higher than the control. By the 3rd day, there is a sharp increase in the hydrolysis products in the prototype. This is since the presence of a significant amount of protein compounds in wastewater contributed to the activation of proteolytic enzymes of the microbiome. The degree of hydrolysis increased by 28.6 times compared to the control. The maximum degree of hydrolysis was noted on day 7 and amounted to 77.1 % in the experimental and 19% in the control samples. Then there is a decrease in the degree of hydrolysis, which is probably due to the further decomposition of organic waste.
Soft treatment modes of wastewater pollution allowed us to obtain a product of high biological value, while maintaining a full-fledged amino acid composition (figure 2).

Figure 1. Degree of hydrolysis.

Soft treatment modes of wastewater pollution allowed us to obtain a product of high biological value, while maintaining a full-fledged amino acid composition (figure 2).

Figure 2. Amino acid composition of the biostimulator.

Table 2. Comparative evaluation of a biostimulator from wastewater with known plant growth stimulators.

| Biostimulator          | Content of free amino acids,% | Glutamic acid,% | Lysine,% | Glycine,% |
|------------------------|-------------------------------|-----------------|----------|----------|
| Raikat Start           | 4                             | 0.96            | 0.56     | 0.48     |
| Aminokat               | 10                            | 2.4             | 1.4      | 1.2      |
| Aminosol Plus          | 59                            | 6.2             | 3.5      | 3.5      |
| Waste water biostimulator | 92.52                        | 11.11           | 5.53     | 9.37     |
Table 3. Effect of the biostimulator on the germination energy and laboratory germination of Scots pine (Pinus Sylvestris).

| Repeat experience | The number of seedlings in the day, pieces | Germination energy, % | Laboratory germination, % | The number of non-germinated seeds, pieces | The number of non-germinated seeds, pieces | Infected with pests |
|-------------------|------------------------------------------|-----------------------|--------------------------|-------------------------------------------|-------------------------------------------|------------------|
|                   | 5 day 7 day 10 day 12 day                 | 6 7 8 9 10 11 12 13 14 | Control 1 2 3 4 5 6 7 8 9 10 11 12 13 14 | Waste water biostimulator 1 2 3 4 5 6 7 8 9 10 11 12 13 14 | Raikat Start 1 2 3 4 5 6 7 8 9 10 11 12 13 14 | Aminokat 1 2 3 4 5 6 7 8 9 10 11 12 13 14 | Aminosol Plus 1 2 3 4 5 6 7 8 9 10 11 12 13 14 |
|                   | 16 14 4 5                               | 68 78 11 3 – 6 2 – 78 | 21 16 5 4 84 94 3 – 1 – 2 – 94 | 2 4 9 10 4 86 94 3 – – 1 2 – 94 | 1 2 10 11 11 66 86 7 2 2 1 – 86 | 13 15 11 7 76 92 4 1 1 – 3 – 92 |
|                   | 16 15 6 5                               | 74 84 8 2 – 5 1 – 84 | 16 15 6 5 74 84 8 2 – 5 1 – 84 | 16 15 6 5 74 84 8 2 – 5 1 – 84 | 16 15 6 5 74 84 8 2 – 5 1 – 84 | 16 15 6 5 74 84 8 2 – 5 1 – 84 |
| Average value     | 12.3 15.3 4.6 5.3                        | 71.3 82 9 2.3 – 5.3 1.3 – 82 | 22.6 10.3 9 3.6 85.3 93.3 3.3 0.3 0.6 0.3 2 – 93.3 | 13.6 11.6 10.6 8.3 72 88 6 1.6 2.3 0.6 1.3 – 88 | 14 13.3 10.6 7 76 90 5 1.6 1 1.3 1.3 – 90 | 13 15.6 10.3 6.6 78 91.3 4.3 1.3 0.3 1.3 1.3 – 91.3 |

Under the action of the enzyme systems of the microbiome, a deep destruction of wastewater proteins occurs. The content of free amino acids was 92.52%. 17 amino acids were found in the test.
product. While in the well-known preparation with an increased content of amino acids, Aminofol Plus contains 9 amino acids (Tyrosine, Arginine, Alanine, Lysine, Proline, Serine, Threonine, Valine and Glutamic acid). The content of free amino acids is 59%. In foreign analogues: Raikat Start, Aminokat-free amino acids 4 and 10%, respectively. It should be noted that the content of glutamic acid, lysine and glycine, which are declared as the main ones in foreign analogues, the resulting biostimulator exceeds several times (table 2).

Since the resulting biostimulator contains a high content of glutamic acid, which activates seed germination, we conducted studies on the effect of the biostimulator on the germination energy and laboratory germination of seeds of scots pine (Pinus Sylvestris). In our experiments, we observed that the use of an amino acid biostimulator improved all the characteristics of seed germination. Treatment of seeds with the obtained amino acid hydrolysate increased germination by 11.3% in relation to the control, germination energy-by 14%, while the number of non-germinated seeds decreased by 2.7 times (table 3). This may be due to the positive role of amino acids in the mobilization of nutrients. The results obtained by us confirm the studies of other scientists about the significant influence of free amino acids on the work of the synthesizing apparatus of plants, participation in the regulation of metabolism [11,15,16].

It should be noted that the treatment of seeds with Raikat Start, Aminokat and Aminofol Plus also contributed to an increase in germination and seed germination energy. But it was lower compared to the proposed biostimulant by 5.3%, 3.3% and 2%, respectively. Perhaps this is due to the method of obtaining a biostimulator. Enzymatic hydrolysis allows you to save the largest amount of amino acids. This in turn affects the formation of peptides in the plant-elements of signaling proteins that perform regulatory functions.

4. Conclusion
The results of the study of the degree of hydrolysis and amino acid composition of fermented meat processing waste show the possibility of their use for a waste-free processing process to obtain biostimulants.

According to the results of the study of the effect of the biostimulator on the sowing qualities of scots pine seeds, it was found that the seeds previously soaked in the biostimulator have better germination and germination energy compared to the control. It is established that microbial fermentation of protein pollutants of wastewater allows to obtain a biostimulator with a more complete set of amino acids. The resulting amino acid biostimulator increased the level of seed shelf life by 11.3%.

The biostimulator helped to improve the quality class of seeds, which allows to reduce the seeding rate per unit area during sowing and, as a result, reduce costs.

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