Study of products influence of rice waste liquid-phase catalytic oxidation on growth and plant development

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Abstract. A method for producing growth stimulants using liquid-phase catalytic oxidation of rice waste was tested, with an assessment of the composition of the resulting solution, active silicon, and bioassay. Laboratory studies have shown that solutions from rice waste synthesized by liquid-phase catalytic oxidation are promising for the preparation of plant growth stimulants.

1. Introduction

Biostimulants are substances and/or microorganisms that, when processing plants, seeds or the rhizosphere, can enhance the growth and productivity of plants and improve crop quality by increasing the availability of nutrients for their absorption, as well as influencing the processes of metabolism and activation of the plant’s protective system [1]. Biostimulants are usually used to treat directly plants or seeds in doses from a few grams to several kilograms per hectare or ton of seeds [2]. Modern biostimulants are mainly represented by humic/fulvic acids, vitamins, amino acids, microbial preparations, seaweed extracts, as well as inorganic substances [1]. Numerous studies indicate the ability of concentrated monosilicic acid to have a stimulating effect on the growth and development of plants [3, 4, 5].

The use of Si-containing compounds increased the yield of crops by 5–50% or more. Although the role of Si in plant physiology remains insufficiently studied, research results indicate an increase in plant viability under the influence of an element [6]. The positive effect of Si on plants is more pronounced under unfavorable growth conditions. The introduction of Si compounds allows one to control the transport system of plants, as well as the permeability of the plasma membrane in leaf cells [7]. Silicon-treated plants demonstrated enhanced chemical stability of DNA, RNA, and chlorophyll molecules, enhanced photosynthesis, and increased biosynthesis of plastid pigments [8, 3]. Under stress conditions, additional Si nutrition provided a decrease in the activity of oxidative degradation processes due to an increase in the content of specific and non-specific antioxidants [9]. It has been hypothesized that Si plays a signaling role in the response of plants to stress [4].

Since rice plants are silicophiles and are characterized by a high Si content (5–10%), it was suggested that it was possible to use the waste small pieces of rice grains remaining after threshing as a biostimulant.

We tested a method for producing growth stimulants using liquid-phase catalytic oxidation of rice waste, studied its composition and active silicon content, and evaluated the effect of watercress on the germination and germination of plants.
2. Materials and methods

2.1. Synthesis of Plant Growth Stimulator
For the synthesis of a plant growth stimulator, the method of liquid-phase catalytic oxidation by air of plant materials was used. Earlier, this method was used to obtain highly effective flame retardant compounds. Rice waste was used as plant material. During the oxidation process, a constant temperature of 60-70 °C was maintained. The oxygen pressure in the reactor was maintained at 3 atm. The end time of the oxidation reaction was determined by the concentration of free alkali by titration with 0.1 N HCl in solution up to 0.5 - 0.8 M. The reaction mixture was filtered on a nylon filter to separate from solid impurities. Thick cream-colored solutions were obtained having an alkaline medium - pH 12.3-12.7.

2.2. Mass spectrometric study
Bond Elut PPL cartridges (Agilent Technologies) (100 mg, 3 ml) were used to desalinate the oxidized samples. Samples were isolated according to the method developed for natural organic matter [2]. Briefly, 5 ml of the concentrated solution was passed through a 0.2 μm membrane filter followed by 5-fold dilution to avoid possible aggregation and gelation. The stained solutions were acidified with 1M HCl to pH 3, followed by passing through a PPL cartridge at ca. 5 ml * min-1. Then, the sorbed material was washed 3 times with 0.01 m HCl to remove most cations, after which the sorbent was thoroughly dried under vacuum. Elution of desalted samples was carried out with a 1.5% solution of ammonia in methanol. Methanol solutions were directly analyzed by FTICR MS.

All experiments were run on a FT MS Bruker Apex Ultra mass spectrometer equipped with a harmonized cell1,5 (Bruker Daltonics), 7 T superconducting magnet, and electrospray ion source (ESI) in negative ionization mode. Before analysis, the test samples were diluted with methanol to a concentration of 100 mg/l. The solutions were injected into the ESI source using a microliter pump with a flow rate of 90 μl/h at a spray gas pressure of 138 kPa and a drying gas pressure of 103 kPa. To ensure rapid evaporation in the ionized droplets, the temperature of the source heater was maintained at 200 °C. Mass spectra were subjected to external and internal calibrations: the first was carried out using synthetic carboxylated polystyrene, and the second, using known residual peaks of fatty acids [6]. The resulting average mass determination accuracy was <0.2 ppm. Spectra were obtained with a data size of 4 megawords ESI(−) and 300 scans were accumulated for each spectrum. The resolution was 530,000 at m/z = 400. The FTICR MS data was processed using the open-source UltraMassExplorer browser application created by Leefmann et al. [8]. The calculated CHONS formulas were verified by applying a chemical filter characteristic of natural organic matter: (O/C ratio ≤ 1, 0.3<H/C ratio ≤ 2.2, atomic restrictions (C ≤ 120, H ≤ 200, 0< O ≤ 60, N ≤ 2, S ≤ 1) and mass accuracy <0.5 ppm). The assigned CHNOS formulas were further plotted on van Krevelen diagrams, which are projections on the axis of the H/C ratio and O/C ratio.

2.3. Plant Impact Assessment
Testing of the obtained preparations was carried out under the conditions of a short-term experiment on watercress seeds (Lepidium sativum L.) [10]. This test is quick, informative and technically simple. Watercress as a test object is convenient in that the effects of stressors and stimulants can be studied simultaneously on a large number of plants with a small area (Petri dishes, etc.).

Testing of drug solutions was carried out in a series of dilutions with distilled water 10⁻³, 10⁻⁶, 10⁻⁹. Solutions in the amount of 10 ml were poured into Petri dishes, filter paper was placed, 30 cress seeds were distributed on it per option, including control. Petri dishes in those replicates were placed in a climatic chamber, where the temperature was maintained at +22 °C and illumination of 130 μmol of protons m² s⁻¹. After 120 hours, the number of seedlings was counted, the length of the roots and coleoptiles was measured. The average values for each option are calculated and the standard deviation is determined.
2.4. Determination of active silicon
To determine the content of monosilicic acid, a modified method of Mullin and Riley was used [11]. The modification consisted of replacing unstable naphthol with iron sulfate and changing the reaction time of monosilicic acid with molybdenum acid ammonium [12].

3. Results
3.1. Identification of synthesis products using mass spectrometry
Thousands of molecular formulas were allowed in the mass spectra of the studied samples, which is typical for products obtained by oxidative condensation [12]. In the samples, the dominance of N-containing compounds is noticeable, which is in good agreement with the peptide content in these samples (Figure 1).

![Figure 1](image_url)

**Figure 1.** A) the amount and B) the relative content of different classes of compounds determined by high resolution mass spectrometry.

The traditional way to visualize data is the Van Krevelen diagram (Figure 2). From the data presented in the diagram, it can be seen that in the studied solutions obtained by liquid-phase catalytic oxidation, the distribution of compounds is characteristic of samples of biological origin. For such samples, the high density of compounds with H/C <1 is atypical [13]. Here, relatively reduced unsaturated compounds with O/C <0.5 and H/C> 1 are characterized by the highest density. It would also like to note the absence of aliphatic oxidized compounds in the products of oxidation.
3.2. Testing on seedlings
It was found that diluted synthesized solutions had a pronounced stimulating effect on the growth of plant roots (Figure 3).
plants was 44.1 mm, 30% more relative to the control. The effect on the length of the seedlings was not fixed, and with a dilution of 10^{-3} it was almost equal - 18.3 mm at 18.4 mm in the control. With a dilution of 10^{-9} relative to the control, the length of the seedlings was 10% less, but stronger roots and intensely colored shoots were visually recorded, that is, there were no signs of plant inhibition.

3.3. Determination of active silicon
The average value of the content of active Si (monosilicic acid) in the synthesized preparations was 335 ± 10 mg l^{-1}. In commercial Si-containing biostimulants, the concentration of monosilicic acid usually ranges from 100 to 500 mg l^{-1} Si. Thus, the preparation we obtained in terms of the content of active silicon can be qualified as a silicon plant growth stimulator.

4. Conclusion
Laboratory studies have shown that solutions of rice synthesized by liquid-phase catalytic oxidation are promising for the preparation of plant growth stimulants. Testing on watercress seedlings showed that the stimulating growth of plant roots is maximally manifested when diluted from 10^{-3}. Testing on other types of plants, in growing experiments, will reveal the optimal dose of the drug, as well as determine the most effective technology for the preparation of the drug.

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