Biological synthesis of bimetallic nanoparticles of cobalt ferrate CoFe2O4 in an aqueous extract of Petroselinum crispum

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Abstract. Synthesized CoFe2O4 nanopowders in an aqueous extract from the leaves of Petroselinum crispum ("Green" synthesis) in an acidic and alkaline medium. Physical and chemical certification of the synthesized nanomaterials by UV spectrophotometry and scanning electron microscopy (SEM) was carried out. According to the UV spectra of CoFe2O4, obtained in an acidic medium, had a small shoulder at 242 nm and a peak at 292 nm, while the particles of alkaline nature had a plateau starting from 229 nm and a peak at 366 nm. SEM found that the synthesis environment also affects the morphology and size of CoFe2O4 bimetallic powders: during the synthesis in an alkaline medium, the particles were hemispherical in size from 30 to 100 nm, and in a slightly acidic medium (pH = 2), larger particles with cut diamond.

The biological activity of cobalt ferrate was shown on the tolerance dynamics of Triticum vulgare L. plants (wheat). Wheat plants were grown in a hydroponic medium with the addition of CoFe2O4 nanoparticles in the range from 10−5 to 10−1 M to 14 days. CoFe2O4 powders of an acidic nature at concentrations of 10−2, 10−3, and 10−5 M reliably increased seed germination relative to intact samples by 48, 37, and 11%, respectively. In turn, CoFe2O4, synthesized in an alkaline medium, increased the index at dilutions of 10−3 and 10−4 M to 4 and 28%.

A detailed analysis of metric indicators of T. vulgare seedlings after 14 days of exposure to CoFe2O4 allowed us to establish a higher resistance of test plants to nanopowders synthesized in an acidic medium. So, when exposed to particles synthesized in an acidic medium, in concentrations from 10−1 M to 10−5 M, an increase in the plant tolerance index of plants from 4 to 14% of the control was recorded. Alkaline CoFe2O4, under the same conditions, increased the rate from 1.2 to 16%.

The intensity of the effect on the pigment apparatus of T. vulgare plants also depended on the synthesis conditions for CoFe2O4: at pH = 2, the amount of chlorophyll a decreased by no more than 2 times relative to the control, at pH = 9 - from 2 to 5 times (P≤0,05).
1. Introduction
Biological, or "green" synthesis of nanoparticles using plants and plant extracts is an attractive alternative to traditional chemical synthesis and more complex methods of cultivation and isolation, necessary for many microorganisms [1]. Among the family of ferrites, cobalt ferrite (CoFe2O4) with excellent physicochemical stability, high anisotropy and magnetization [2] is one of the most interesting [2], which makes it a suitable candidate for biomedical applications [3, 4]. Earlier, the possibility of high antimicrobial activity of CoFe2O4 was shown [5]. However, very few studies have been devoted to the biological effects of biosynthesized nanoparticles on plants [6], and there is almost no work in this aspect on bimetallic nanopowders. In accordance with the desire to achieve greater understanding in this regard, we analyzed the result of the interaction of cobalt ferrate nanopowders, obtained by the method of green synthesis, with plant seedlings.

2. Materials and Methods

Synthesis of nanoparticles of cobalt ferrate. Fresh vegetable raw parsley Petroselinum crispum washed under running water, then twice with distilled water, dried on filter paper to remove moisture from the surface of the leaves. Then crushed using liquid nitrogen to a powder, was added to 1 part of the extract 2.5 parts of distilled water (by weight). Then heated for 30 minutes at a temperature of 90 ° C. After heating, it was filtered through 2 layers of gauze to remove plant residues. Purified extract by centrifugation at 15,000 vol. within 15 min the resulting supernatant was filtered through millipore with a pore diameter of 0.45 μm. The resulting purified extract was diluted with distilled water (1: 3) for further use in the synthesis [7].

The initiation of the synthesis consisted in the preliminary dissolution of the precursor salts — 7 g CoCl2 · 6H2O and 13.9 g FeCl3 · 6H2O - in 200 ml of extract (pH = 2) and constantly stirring at 100 ° C for 6 h (before xerogel formation). Then the liquid was evaporated, and the gel was washed with distilled water (2 times) and alcohol (1 time) by centrifuging at maximum speed for 10-15 minutes. Then the gel was dried in a thermostat at 200-300 ° C for 3 h and annealed at 800 for 1 h.

Synthesis of CoFe2O4 in an alkaline medium was distinguished by the fact that 1 M NaOH was added dropwise to a solution of ferrous sulfate with constant stirring until the pH ≥ 9 was determined titrimetrically.

Characterization of nanoparticles. The obtained particles CoFe2O4 were resuspended in distilled water in an amount of 1 g/l, the suspension was treated in an Elmasonic ultrasonic bath for 15 min, and characterized using UV/vis-spectrophotometry, scanning electron microscopy (SEM) и методом динамического светорассеяния.

UV-spectrophotometry. A suspension of 0.1 M NPs CoFe2O4 and a solution of salt were analyzed spectrophotometrically in the wavelength range 200-600 nm in quartz cuvettes with an optical path length of 1 mm. Water was used as a reference solution. The efficiency of the synthesis of NPs was analyzed by integrating the UV-spectra (SF-2000) of salt and the supernatant. The supernatant was obtained by mixing the salt and the extract and then filtering the mixture through a filter (0.22 μm).

SEM. The nanoparticles CoFe2O4 were deposited onto a double-sided adhesive carbon tape (2SPI, USA) and examined with a Zeiss Merlin microscope equipped with Gemini II Electron Optics (Zeiss, Oberkochen, Germany). The measurements were carried out at accelerating voltage of 1-5 kV and probe current 25-80 pA without any conductive coating on the sample surface.

Evaluation of biological activity of nanoparticles. To assess the biological activity of the synthesized CoFe2O4 powders, seeds of a standard test plant Triticum vulgare were taken, previously they were disinfected and germinated in a climatic chamber (Agilent, USA) under 12-hour illumination, temperature 22 ± 1°C and humidity 80 ± 5% for 48 hours. Then equally germinated seeds (20 pieces) were transferred to individual cups and 5 ml of NPM suspensions were added in concentrations from 10-1 to 10-5 M. Samples were left to germinate for 7 and 14 days and then measured the length of the leaves and roots of seedlings. The percentage of germination energy (E) was calculated on the 3rd day in the formulation, when the seeds immediately after soaking were watered with 5 ml of particle
suspensions. At the end of the experiment, the length of the roots and leaves of seedlings was measured, and the content of photosynthetic pigments, OP (chlorophyll a, b, and carotenoids) in the ethanol extract was also evaluated by spectrophotometric method [8]. Next, the tolerance index (IT), corresponding to the ratio of the effective average value in seedlings in the presence of NP, to the average indicator of control seedlings in% was evaluated. Accordingly, the more IT, the greater the resistance of plants and the lower the toxicity of NPM, and vice versa. For fewer stable seedlings were taken those whose IT was less than 50% compared with the control. The method of plant germination and the evaluation of the biological activity of nanopreparations on T. vulgare is described in more detail in our previous works on physico-chemical nature nanomaterials [9-11]. All experiments were performed in 3 replications. The obtained data was processed using Microsoft Excel and Statistica V8.

3. Results and its discussion

UV spectrophotometry showed differences in the spectra of nano-CoFe2O4 synthesized under various conditions. Thus, in an acidic medium, the nanopreparation had a small shoulder at 242 nm and a peak at 292 nm, while particles of an alkaline nature were characterized by a plateau, starting at 229 nm and a peak at 366 nm (Fig. 1). SEM found that the synthesis environment also affects the morphology and size of CoFe2O4 bimetallic powders. In particular, in the synthesis of CoFe2O4 in an alkaline medium (pH\geq9), the particles were obtained smaller (from 30 to 100 nm) and almost did not have a cut (Fig. 2 A). In turn, in a weakly acidic medium (pH = 2), large particles with a characteristic facet were formed and, possibly, were single crystal (Figure 2 B).

![Figure 1. UV spectra of CoFe2O4 nanoparticles synthesized in an aqueous extract from P. crispum leaves](image-url)
Figure 2. Scanning electron microscopy of bimetallic CoFe$_2$O$_4$ nanoparticles synthesized in an aqueous extract from P. crispum leaves: A - synthesis at pH$\geq$9, B - synthesis at pH = 2, bar - 100 nm

An analysis of the biological activity of the synthesized CoFe$_2$O$_4$ particles by the example of the test organism T. vulgare showed a difference in the germination energy and morphometry of seedlings, depending on both the concentration of the exogenous agent and the environment of their synthesis. Acidic powders (pH = 2) at concentrations of $10^{-2}$, $10^{-3}$, and $10^{-5}$ M reliably increased seed germination relative to intact samples by 48, 37, and 11%, respectively (Fig. 3). In turn, CoFe$_2$O$_4$, synthesized in an alkaline medium, increased the index at dilutions of $10^{-3}$ and $10^{-4}$ M to 4 and 28% ($P \leq 0.05$).

A detailed analysis of the metric indices of T. vulgare seedlings after 14 days of exposure to CoFe$_2$O$_4$ allowed us to establish a higher resistance of test plants to nanopowders synthesized in an acidic medium (Fig. 4). Thus, an increase in the concentration of particles synthesized in an acidic medium from $10^{-1}$ M to $10^{-5}$ M contributed to an increase in the index for leaves from 4 to 14% of the control. At the same time, CoFe$_2$O$_4$ of alkaline nature in the same conditions increased the rate from 1.2 to 16%.

Figure 3. The effect of bimetallic nanoparticles obtained by a biological method on the germination energy of T. vulgare seeds
Figure 4. The values of the upper limit of the tolerance index (%) according to growth indicators of T. vulgare after treatment with bimetallic CoFe2O4 nanoparticles obtained by a biological method.

The effect on the pigment apparatus of T. vulgare plants also depended on the synthesis conditions and the concentration of metals. CoFe2O4 NPs synthesized at pH = 2 affected the content of AF to a lesser extent, reducing the amount of chlorophylls (not more than 2 times relative to the control, respectively) (Fig. 5). On the contrary, the preparations obtained in an alkaline medium led to a noticeable decrease in the level of chlorophyll a - the index decreased from 2 to 5 times (P≤0.05).

Figure 5. The influence of CoFe2O4 nanoparticles obtained by a biological method on the content of photosynthetic pigments in the leaves of T. vulgare plants.
Thus, the combination of cobalt with iron led to a concentration-dependent change in the resistance of the test plants *T. vulgare* — high concentrations (10-1 and 10-2 M) resulted in slight damage to the plant cells, and in small concentrations (less than 10-3 M) drugs stimulated seed germination and seedling growth.

However, the difference in biological activity was also associated with the conditions of particle synthesis - the alkaline environment led to a lower tolerance of plants. Probably, ferrite nanoparticles under different conditions of medium pH include various mechanisms of inversion of Co2 + and Fe3 + ions on the surface of particles [12]. It is known that in an acidic medium, the processes of metal ion extraction from an NP core are enhanced more [13]. Despite this, CoFe2O4, synthesized at pH = 10, probably led to the release of more Co2 + [14] and ROS [15, 16]. Of course, functional groups could have a direct effect on the outcome of the toxicity of the NPs under study (after calcining the powder, the organic phases of the extract can remain in the form of –COOH, –OH, and –NH2 groups), which was confirmed in a study [17].

The difference in the manifestation of biological effects could also be due to the size and character of the surface of the particles, due to the method of synthesis. According to the SEM images of CoFe2O4, synthesized at pH = 10, although they were spherical and oval formations, they had a smaller size compared to particles obtained at pH = 2, which, even considering more sharp edges [18], had smaller inhibitory properties on plants.

4. Conclusion
Despite the stable IT of seedlings to cobalt ferrate, a slight decrease in chlorophyll a leaves (especially in the case of CoFe2O synthesized at pH = 10) could be due to a slight redox imbalance [10, 11, 19] and a malfunction of Photosystem I [12], which did not affect the decrease in leaf growth at all. The data obtained by us are consistent with the work [20], in which, by the example of tomato, it was shown that plants are able to tolerate CoFe2O4 NP concentrations up to 1000 mg / l without visible signs of toxicity.

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