A new method for the selenium nanoparticles synthesis and study of their influence on the characteristics of physiological processes in spring rye seedlings at the initial stages of ontogenesis

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Abstract. A new method for the synthesis of selenium nanoparticles based on its organic compound is described, which makes it possible to obtain nanoparticles with a size of 1.8-3.5 nm. It was established that the growth processes and development of spring rye plants were stimulated by adding the selenium nanoparticles to the growing medium up to 91% and 25% as compared to the control and other selenium-containing preparations, accordingly.

1. Introduction
Selenium is an indispensable trace element in the life of living organisms, therefore, the search of their new forms for the delivery to the body is an urgent task. Selenium compounds can be used as growth stimulants, antioxidants and restorers of enzymatic functions [1-3]. Selenium is an essential trace element as it takes part in many cellular processes [2].

Elemental selenium is generally considered biologically inert since it is practically insoluble in water [1]. However, in recent years it was found that selenium nanoparticles can be absorbed by living organisms [4-7]. It should be considered that selenium particles, which are micro-sized, can be toxic to some microorganisms [8]. In addition, the use of nanoparticles to control the incidence of plants and antimicrobial mechanisms in the plant ecosystem have certain prospects [9].

In Russia, intensive work is underway on the use of nanoparticles in crop production. This is evidenced by a review of studies carried out in research and academic institutes in Russia that use nanoparticles of metals (Fe, Cu, Co and Ag) and non-metals (phosphorus, silicon, selenium, and bischofite) with sizes from 1 to 100 nm in the form of aqueous suspensions and colloidal solutions. The main seed treatment method used is soaking. Typically, exposure to nanoparticles has a positive effect on the germination and yield of plants. Cereals were used as the main experimental crops. The review summarizes the results of processing vegetables and oilseeds. The yield increase ranged from 0.4 to 45 centners per hectare; seed germination increases in the range of 10-20%. After treatment with nanoparticles, there are some improvements in the biochemical composition of the cultures [10].

There are a number of principles for the synthesis of selenium nanoparticles. The most widely used methods of synthesis are based on the reduction of oxidized forms of inorganic selenium. Nanoparticles of various shapes and sizes can be synthesized from selenium salts, especially selenites and selenates in the presence of reducing agents, such as proteins, phenols, alcohols, and amines, but the most interesting at this point in time are biogenic syntheses of such particles [11].
The synthesis of selenium nanoparticles based on organoselenium compounds has been realized only in recent years using biotechnological methods [12-13]. It should be noted that biotechnological synthesis can also be used to obtain selenium nanoparticles from inorganic raw materials [14-15].

The aims of this work were: the search for methods for the synthesis of selenium nanoparticles of not more than 10 nm in size and the study of growth and physiological processes in spring rye seedlings at the initial stages of ontogenesis under the influence of these particles.

It was decided to synthesize selenium nanoparticles on the basis of DAFS-25k, which forms complex compounds or compounds oxidized at the selenium atom when interacting with Lewis acids or phosphorus pentachloride [16-17], however, like all selenium esters, it should decompose under the action of Lewis bases [18].

2. Materials and methods

Diacetophenonylselenide (preparation DAFS-25) I was taken as reference drugs due to the availability of literature data on its use in this aspect [19-22] and 2,6-diphenyl-4-(p-dimethylaminophenyl)-4H-selenopyran II - new compound of the heterocyclic series. Heterocyclic compounds in this aspect have also been used (figure 1) [23-25].

![Heterocyclic compounds.](image1)

Figure 1. Heterocyclic compounds.

For the synthesis of selenium nanoparticles, diacetophenonyl selenide (DAFS-25 preparation) was chosen as a selenium-containing substrate, polyvinylpyrrolidone was chosen as an adsorbent for selenium nanoparticles, and isopropanol was used as a solvent (figure 2).

![Synthesis of selenium nanoparticles.](image2)

Figure 2. Synthesis of selenium nanoparticles.

Experimental procedure for the synthesis of selenium nanoparticles. Mix 6.34 g of polyvinylpyrrolidone powder (PVP, Sigma Aldrich) with 50 ml of isopropanol preheated to 65 °C. Mix gradually, as the vapor of heated isopropanol acts on PVP, which leads to sticking of the substance. Next, 3.17 g of DAFS-25k preparation is added to the flask and stirred until complete dissolution. Then, 3.4 ml of 25% ammonia solution was added to initiate the reaction. The process continues until the yellow color of the flask contents stabilizes. Then the contents of the flask are cooled to stop the reaction. After that, the reaction mixture is placed in a freezer at a temperature of -53 °C for freeze-drying.

3. Results and Discussion

The resulting mass is analyzed for selenium content. The result is 10%, which is slightly lower than the theoretical one (11%). This indicates that during the decomposition of the DAFS-25 preparation, not only volatile impurities (for example, acetophenone) were obtained, but also other reaction products.

The most important aspect of the studies performed was the size of the obtained selenium nanoparticles. Determination of the size of selenium nanoparticles was carried out on a Zeiss Libra
120 transmission electron microscope (Germany) on the basis of the Center for Shared Use of Scientific Equipment in Physicochemical Biology and Nanobiotechnology "Symbiosis" of the Federal State Budgetary Institution of Science, Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences (Saratov). Qualitative analysis for the selenium content in the studied samples was carried out using the EELS method (electron loss spectroscopy).

The size of selenium nanoparticles (mostly) was in the range of 1.8-3.5 nm (figures 3-5), which is quite acceptable for subsequent biological experiments, since it contributes to a greater percentage of selenium assimilation by living organisms.

Figure 3. Selenium nanoparticles in freeze-dried reaction mass.

Figure 4. Selenium nanoparticles in freeze-dried reaction mass.

Figure 5. Selenium nanoparticles in freeze-dried reaction mass.
Further, it was decided to test for biological activity the resulting system containing 10% selenium nanoparticles (by weight) using the example of rye seeds. For this, five groups of 50 rye seeds of the spring variety Volzhanka were made up.

Before starting the experiment, solutions of organoselenium preparations are prepared by dissolving: 0.125 g of nanoselenium, 0.063 g of 2,6-diphenyl-4- (p-dimethylamino) -4H-selenopyran, and 0.05 g of DAFS-25 in 5 ml of isopropanol each. The resulting solutions correspond to the content of elemental selenium at a concentration of 2.5 mg/ml.

For biological experiments, solutions are prepared in distilled water, which are used in further experiments.

Solutions:

- Distilled water (50 ml);
- Distilled water (50 ml) + 0.5 ml of a solution of 2,6-diphenyl-4- (p-dimethylamino) -4H-selenopyran;
- Distilled water (50 ml) + 0.5 ml of nanoselenium solution;
- Distilled water (50 ml) + isopropium alcohol (0.5 ml);
- Distilled water (50 ml) + 0.5 ml of DAFS-25 solution.

In flat-bottomed flasks add 50 seeds of spring rye and fill them with prepared solutions. The seeds are soaked for 24 hours by placing the flasks on a shaking apparatus - a shaker.

Spread the seeds on a filter paper and dry for 30 minutes.

Then spread the seeds evenly on a strip of filter paper moistened with distilled water and wrap it in a roll. Rolls are placed on Petri dishes and placed in an oven at a temperature of 25-30 °C. Every 1-2 days the seeds are moistened with distilled water.

We carried out studies on the possibility of growth and physiological changes in the growth processes of spring rye when using selenium-containing substances, using the example of 2,6-diphenyl-4- (p-dimethylamino) -4H-selenopyran, selenium nanoparticles and diacetophenonyl selenide (DAFS-25).

When analyzing the daily dynamics of growth, it was found that the solution with the addition of nanoselen has the greatest growth-stimulating activity (figure 6).

![Figure 6. Daily dynamics of growth of spring rye seeds.](image-url)

The general dynamics of growth also proves that selenium-containing compounds have a beneficial effect on the growth and development of spring rye seeds (figure 7).
The dynamics of seed growth using selenium nanoparticles shows an increase in the dynamics of seed growth by 91% relative to water and by ~ 25% relative to 2,6-diphenyl-4- (p-dimethylamino) -4H-selenopyran and diacetophenonyl selenide (DAFS-25 preparation).

4. Conclusion
The features of growth and physiological processes in spring rye seedlings at the initial stages of ontogenesis under the influence of growth regulators were revealed. It has been established that the growth processes and development of spring rye plants are stimulated by adding 91% selenium nanoparticles to the growing medium relative to water. An increase in the dynamics of seed growth by ~ 25% was revealed for dimethylselenopyran, a solution of isopropyl alcohol in water, and diacetophenonyl selenide.

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