Dispersion methods of preparation of nanosized biological agents for vegetable crops

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Abstract. The effect of iron and selenium colloidal solutions on seeds was studied. The obtained results showed a different reaction of plants to treatment with solutions of nanosized iron and selenium. Onion seeds in both cases showed low germination – 15%. Colloidal iron treatment showed 100% germination of zucchini and radish seeds as in control, but an iron concentration of 10 mg/l inhibits plants. The greatest root length for beets, cucumbers and carrots was noted at a dose of nanosized iron of 1 mg/l. The root length in the experimental and control samples of zucchini and radish do not differ within the error limits. An increase in seed germination during treatment with nanosized selenium compared with the control samples was observed in onions, carrots, cucumbers, turnips, pumpkins and clarkia. Seed germination of aster was 15%, while in the control sample germination was absent. Nanopowders stimulate the processes of adaptation and self-organization of biological systems and reduce the negative impact of adverse environmental factors.

1. Introduction
Nanoscale materials have unique properties, including biological activity. A method has been developed for pre-sowing seed preparation by exposure to a colloidal solution of small metal particles, in particular, involving the treatment of iron nanoparticles [1]. They accelerate seed germination, stimulate the stages of plant development until the end of the growing season. Seed treatment with colloidal metal solutions during sowing does not affect soil performance, the substance does not accumulate in it, which indicates the absence of cumulative properties of the preparation used and its environmental safety [2]. The author of the cited work suggested that nanoscale substances change the activity of enzymes, proteins, vitamins, and other biologically active substances. There is reason to believe that the substance in colloidal form penetrates the cells, gives up stored energy, contributing to the germination and development of plants. The presence of physiologically active compounds in plant foods improves their nutritional value by performing biochemical functions in animals. Processing with nanosized particles spreads the effect on the second generation of plants.

A number of methods for producing biologically active nanoscale materials were developed. Among them, metals such as iron, copper, cobalt, and non-metals – selenium and silicon. Experimental batches of such materials were made and on their basis, experimental samples of nanoscale agricultural biological products were prepared and studied.

The aim of the work is the studying of effect on the vegetable crops seeds of biologically active nanoscale iron and selenium colloidal solutions.
2. Materials and Methods

Selenium solution were obtained by laser ablation in an aqueous medium [3]. For the preparation of selenium colloidal solutions, a solid-state laser with a radiation wavelength of 1064 nm, a pulse energy of 2.50 J with a pulse duration of 12 ns was used. The pulse repetition rate is 1 Hz.

Figure 1. AFM of a precipitate of an evaporated selenium colloidal solution on a silicon surface.

Dispersed biologically active nanosized metal powders were obtained by low-temperature hydrogen reduction of artificially prepared raw materials in the form of nanosized metal hydroxide [4, 5]. Obtained powder was dispersed by ultrasonic treatment to colloidal solutions with concentration 0.1, 1.0 and 10.0 mg/l for spraying seeds. The figure 2 shows scheme of iron nanoparticle structure based on high-resolution electron microscopy.

Figure 2. Scheme of iron biologically active nanoparticle:
1 – core of α-Fe, 2 – iron oxides cover, 3 – smaller nanoparticles.

The tests were carried out on the seeds of the following vegetable crops: «Kibray» dill, «Bordeaux 237» beet, «Petrovskaya» turnip, «Chernomor F1» cucumber, «Muscat» pumpkin, «Gribovsky 37» zucchini, «Moscow winter» carrot, pea «Oregon» and asters «Calypso». A physiological saline solution based on bidistilled water was used in the control samples. Treated seeds were laid out in Petri dishes, 20 in each. The percentage of seed germination, the stem and root length of the seedling were determined. The repetition of experience is tenfold. The arithmetic mean and the mean error were calculated [6].

The literature data on the effect of selenium on plant growth and productivity are contradictory [7]. A large number of works are devoted to the study of enrichment of plants with selenium due to its low content in soils. The concentration of selenium was selected based on the work of P. Prudnikov [8], in which he discovered the effect of stimulating plant growth with a solution of sodium selenite with a concentration of $\text{Se} \cdot 2.6 \cdot 10^{-2} \text{ mmol/l}$. 
3. Results and discussions

Germination under the influence of nanosized iron on the third day after the start of the experiment is presented in table 1.

| Species | Control samples (%) | 0.1 mg/l of Fe (%) | 1.0 mg/l of Fe (%) | 10.0 mg/l of Fe (%) |
|---------|---------------------|--------------------|--------------------|--------------------|
| Onion   | 11.0±0.3            | 12.0±0.3           | 13.75±0.4          | 12.4±0.4           |
| Beet    | 70.0±2.8            | 75.0±3.8           | 89.0±3.5           | 85.0±3.0           |
| Turpin  | 90.0±2.1            | 92.0±2.1           | 90.0±3.5           | 92.0±3.1           |
| Cucumber| 85.0±1.5            | 84.0±1.5           | 90.0±2.5           | 89.0±2.4           |
| Pumpkin | 79.0±2.1            | 80.0±2.1           | 92.0±2.8           | 90.0±2.7           |
| Zucchini| 100.0±3.9           | 100.0±4.9          | 100.0±3.9          | 92.0±3.9           |
| Carrot  | 52.0±2.3            | 53.0±2.3           | 76.0±2.5           | 69.0±2.5           |
| Peas    | 79.0±3.6            | 76.9±3.4           | 87.0±3.2           | 89.0±3.2           |
| Radish  | 100.0±4.1           | 100.0±3.9          | 100.0±4.2          | 92.0±4.3           |
| Tomato  | 89.0±3.8            | 89.0±3.7           | 95.0±3.5           | 96.0±3.7           |

Table 1. Seed germination treated by nanoscale iron.

| Species | Control samples (mm) | 0.1 mg/l of Fe (mm) | 1.0 mg/l of Fe (mm) | 10.0 mg/l of Fe (mm) |
|---------|----------------------|---------------------|---------------------|----------------------|
| Onion   | 0                    | 0                   | 1.4±0.09            | 1.3±0.09             |
| Beet    | 10.8±0.15            | 10.4±0.2            | 11.4±0.2            | 10.9±0.3             |
| Turpin  | 34.4±1.5             | 32.4±1.8            | 33.0±2.5            | 30.4±1.8             |
| Cucumber| 59.4±2.3             | 60.4±2.3            | 65.4±2.3            | 62.4±2.2             |
| Pumpkin | 59.4±2.3             | 48.9±1.6            | 47.2±1.5            | 45.0±1.5             |
| Zucchini| 35.7±1.9             | 35.8±1.7            | 35.9±1.6            | 30.7±1.7             |
| Carrot  | 5.1±0.09             | 4.9±0.08            | 5.5±0.1             | 5.3±0.1              |
| Peas    | 28.3±0.7             | 28.5±0.07           | 26.3±0.9            | 28.6±0.1             |
| Radish  | 37.0±1.9             | 36±1.8              | 39±1.7              | 33±1.5               |
| Tomato  | 4.1±0.05             | 3.9±0.1             | 3.5±0.1             | 3.4±0.1              |

Table 2. Root length of seeds treated by nanoscale iron.

Onion seeds showed low germination – less than 15%. Colloidal iron treatment showed 100% germination of zucchini and radish seeds as in control, but an iron concentration of 10 mg/l inhibits plants. Germination of carrots increased by 46% when treated with iron at a concentration of 1.0 mg/l. The remaining seeds reacted to the same concentration. An important factor is the increase of field germination of seeds, associated with the activation of growth, as well as increased plant productivity and their resistance to extreme environmental factors [2].

The root length under the influence of nanoscale iron is given in table 2. Onion root appeared only when treated with nanoscale iron. The greatest root length for beets, cucumbers and carrots was noted at a dose of nanosized iron of 1 mg/l. The root length in the experimental and control samples of zucchini and radish do not differ within the error limits. Turnips, pumpkins, peas and tomatoes have less root in the experiment.
The shoot length under the influence of nanoscale iron is given in table 3.

**Table 3.** Shoot length of seeds treated by nanoscale iron.

| Species    | Control samples (mm) | 0.1 mg/l of Fe (mm) | 1.0 mg/l of Fe (mm) | 10.0 mg/l of Fe (mm) |
|------------|----------------------|---------------------|---------------------|----------------------|
| Onion      | 1.2±0.09             | 1.4±0.09            | 1.4±0.09            | 1.3±0.09             |
| Beet       | 18.0±0.2             | 17.8±0.2            | 20.1±0.4            | 20.2±0.8             |
| Turpin     | 16.6±0.5             | 17.8±0.5            | 16.8±0.5            | 15.6±0.8             |
| Cucumber   | 29.2±1.5             | 29.1±1.5            | 37.1±1.8            | 30.1±1.4             |
| Pumpkin    | 14.0±0.06            | 15.0±0.06           | 15.0±0.06           | 15.0±0.09            |
| Zucchini   | 24.2±1.2             | 24.0±1.2            | 24.8±1.4            | 20.2±1.0             |
| Carrot     | 3.2±0.07             | 3.3±0.06            | 4.8±0.08            | 4.5±0.08             |
| Peas       | 5.4±0.06             | 5.8±0.07            | 6.7±0.09            | 6.5±0.09             |
| Radish     | 24.6±0.9             | 24.0±0.5            | 28.6±0.5            | 23.8±0.1             |
| Tomato     | 3.9±0.05             | 3.8±0.05            | 4.9±0.05            | 4.7±0.1              |

Onion shoot length increased by approximately 16%. Zucchini does not respond to nanoscale iron. Shoot length of the remaining seeds was increased by a concentration of nanoscale iron of 1.0%.

The results showed a different effect of plants on the treatment with a solution of nanosized selenium compared to the control samples was observed in onions, carrots, cucumbers, turnips, pumpkins and clarkia. Seed germination of aster was 15%, while in the control sample germination was absent. Watercress and radish have no significant difference between control and treated samples.

**Table 4.** The effect of nanosized selenium on germination, root length and shoot.

| Species    | Germination (%) | Root length (mm) | Shoot length (mm) |
|------------|-----------------|------------------|-------------------|
|            | Control | Nano Se | Control | Nano Se | Control | Nano Se |
| Onion      | 10±0.3     | 15±0.5   | 0       | 3.3±0.05 | 0       | 1.0±0.09 |
| Dill       | 15±0.6     | 12±0.3   | 1.7±0.05 | 3.0±0.015 | 1.5±0.05 | 2.9±0.015 |
| Beetroot   | 60±1.8     | 42±1.2   | 10.8±0.15 | 10.5±0.15 | 17±0.1 | 14±0.2 |
| Turnip     | 86±2.1     | 95±3.2   | 32.4±1.5 | 24.1±0.8 | 12.6±0.1 | 16±0.1 |
| Cucumber   | 89±1.2     | 100±2.8  | 68.4±2.3 | 68.9±2.0 | 36.2±1.5 | 34.1±1.0 |
| Pumpkin    | 78±2.1     | 86±3.0   | 53.2±1.5 | 65.6±2.5 | 16.0±0.06 | 18.9±1.0 |
| Zucchini   | 100±3.9    | 97±4.5   | 40.7±2.1 | 65.3±2.0 | 27.2±1.0 | 22.4±0.05 |
| Carrot     | 56±2.3     | 78±3.1   | 4.9±0.05 | 2.8±0.09 | 2.8±0.07 | 1.7±0.04 |
| Watercress | 100±1.5    | 100±2.3  | 28.9±1.0 | 20.7±0.8 | 29.2±0.09 | 28.6±0.08 |
| Peas       | 81±3.6     | 46±1.4   | 29.3±0.7 | 21.1±1.1 | 5.8±0.06 | 3.1±0.09 |
| Radish     | 100±4.1    | 100±2.8  | 35±1.8  | 17.4±1.0 | 25.6±0.9 | 23.9±1.0 |
| Aster      | 0         | 15±0.5   | 0       | 2.1±0.03 | 0       | 2.7±0.08 |
| Clarkia    | 22±0.5     | 31±0.7   | 2.5±0.07 | 4.7±0.09 | 7.5±0.05 | 6.7±0.09 |

The results showed a different effect of plants on the treatment with a solution of nanosized selenium.
4. Conclusion
A colloidal solution of iron and selenium has different effects on seed germination, root and shoot length. Growth stimulation takes place for seeds of onion, beetroots, turnip, cucumber, nutmeg, zucchini, carrots and radishes. For watercress, aster and clarkia, only the effect of a colloidal solution of selenium was studied.

The nanosized iron and selenium used in this study with a particle size of 20-100 nm are comparable with the sizes of cell structures. Nanoscale particles probably transmit energy to cellular structures, this can explain their biological and environmental effectiveness. Nanopowders with energy exposure stimulate the processes of adaptation and self-organization of biological systems and reduce the negative impact of adverse environmental factors.

The use of nanotechnology in agricultural production is an effective and reliable way to significantly increase agricultural productivity and the quality of domestic food raw materials.

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References
[1] Folmanis G E The method of presowing preparation of seeds Patent of Russia Federation No. 2056084, BI No. 8, Published 20.03.1996
[2] Churilov G I and Ampleeva L E 2010 The Biological Effect of Nanosized Metals on Various Plant Groups (Ryazan: RSATU) p 150
[3] Folmanis G E, Kovalenko L V, Fedotov M A, Kazilin E E and Kolokoltsev V N 2018 Nanomaterials for agriculture A.A.Baikov’s Institute of Metallurgy and Material Science 80 years (Moscow: Intercontact Science) pp 217-225 DOI: 10.30791/978-5-902063-58-2-217-225
[4] Baldokhin U V, Korneev V P, Kovalenko L V, Suzdalev I P, Kolotyrkin P Ya, Arsent’eva I P and Folmanis G E 2009 Low-temperature hydrogen reduction of iron hydroxide. The formation of nanoclusters and nanostructures Advanced Materials 6 pp. 72-76
[5] Kovalenko L V and Folmanis G E 2006 Biologically active nanopowders of iron (Moscow: Science) p 124
[6] Lakin G F 1990 Biometrics (Moscow: Highest school) p 343
[7] Torshin S P, Udel’nova T M and Yagodin B A 1996 Biogeochemistry and agrochemistry of selenium and methods for eliminating selenium deficiency in food and feed Agrochemistry 8-9 pp. 127-145
[8] Prudnikov P S 2006 The effect of selenium on the work of the antioxidant system in the shoots of the renewal of potato tubers under hypothermia Proc. Int. Conf. of Young Botanists in St. Petersburg (St. Peterburg: GETU «LETI») p 188