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STUDY OF THE BIOLOGICAL ACTIVITY OF ARABINOGALACTAN-STABILIZED SILVER NANOPARTICLES TOWARDS WATERCRESS *Lepidium sativum* L. cv. Curled AND PLANT PATHOGENIC MICROMYCETE *Fusarium sambucinum*

O.I. GUDKOVA[1], N.V. BOBKOVA[1], N.B. FELDMAN[1] ✉, A.N. LUFEROV[1], T.I. GROMOVYKH[1], I.A. SAMYLINA[1], M.A. ANANYAN[2], S.V. LUTSENKO[1]

[1]*Sechenov First Moscow State Medical University (Sechenov University),* the Ministry of Health of the Russian Federation, 8-2, ul. Trubetskaya, Moscow, 119991 Russia, e-mail senia501@yandex.ru, bobkovamma@mail.ru, n_feldman@mail.ru (corresponding author ✉), luferovc@mail.ru, gromovykhtatyana@mail.ru, laznata@mail.ru, svlutsenko57@mail.ru;
[2]*Nanoindustry Concern JSC,* 4-1, ul. Bardina, Moscow, 119334 Russia, e-mail nanotech@nanotech.ru
ORCID:
Gudkova O.I. orcid.org/0000-0003-3880-5587
Bobkova N.V. orcid.org/0000-0003-1591-4019
Feldman N.B. orcid.org/0000-0001-6098-2788
Luferov A.N. orcid.org/0000-0003-2397-7378
Gromovykh T.I. orcid.org/0000-0002-6943-534X
Samylina I.A. orcid.org/0000-0002-4895-0203
Ananyan M.A. orcid.org/0000-0002-1588-1475
Lutsenko S.V. orcid.org/0000-0002-2017-6025

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Abstract

Metal nanoparticles (NPs) exhibiting growth-stimulating, antifungal, antibacterial, insecticidal effects and prolonged release of minerals and herbicides, opens up prospects for increasing the yield of crops. Among metal nanoparticles that can find application in agriculture, silver nanoparticles occupy a special place due to a wide spectrum of biological activity. In this work, we have established for the first time that the pre-sowing treatment of seeds of watercress *Lepidium sativum* L. cv. Curled by silver nanoparticles which are stabilized by the biopolymer arabinogalactan and dioctyl sulfosuccinate affects the germinative energy, laboratory seed germination and some anatomical and morphometric parameters of watercress seedlings. It was shown for the first time that silver nanoparticles have an inhibitory effect on the growth of the phytopathogenic fungus *Fusarium sambucinum*. This work aimed to assess both the stimulating effect of silver nanoparticles (Ag-NPs) stabilized with arabinogalactan and dioctyl sulfosuccinate on growth of watercress *Lepidium sativum* L. cv. Curled seedlings and the antifungal effect on a plant pathogenic toxin-producing micromycete *Fusarium sambucinum* VKPM F-900. The nanoparticles were synthesized by the reduction of silver nitrate in an alkaline medium in the presence of arabinogalactan followed by the addition of dioctyl sulfosuccinate as a stabilizer. The average nanoparticle diameter was 11.40±3.96 nm; zeta potential –24 mV. The effect of silver nanoparticles on germination energy, seed germination, growth of watercress seedling hypocotyl and root was investigated. Seeds were incubated in sols of nanoparticles with various silver concentrations (1.17, 2.34, 4.69, 9.38, 18.75. 37.5, 75, and 150 μg/ml). Control seeds were incubated in water. After incubation, the seeds were germinated in Petri dishes on a wet bed of filter paper in the dark at 20 °C. The seed germination energy was determined on day 3, the laboratory germination — on day 5, the lengths of the hypocotyl and the main root were measured on day 7, and also microscopic analysis of the root sections of seedling treated with sols with stimulating and inhibiting concentrations of Ag (4.69 and 18.75 μg/ml, respectively) was carried out. Antifungal activity of silver sols with concentrations from 9.38 to 300 μg/ml was assessed by the agar diffusion method. Micromycete *Fusarium sambucinum* Fuckel VKPM F-900 was used as a test culture to determine antifungal activity. Sterile water was used as a control. The incubation of seeds in sols with a silver concentration of 2.34 and 4.69 μg/ml had a stimulating effect on the germination energy and laboratory germination of *L. sativum* seeds. A dose of silver nanoparticles of 4.69 μg/ml increased the germination energy by 13.5 % and laboratory germination by 11.7 % compared to the control. In addition, the concentrations of silver from 1.17 to 4.69 μg/ml had a significant stimulating effect on root growth (from 34.4 to 79.1 %, respectively) with some deceleration of hypocotyl growth. Seed incubation in sols with a silver concentration of 18.75 μg/ml and higher led to a significant decrease in the germination energy and laboratory germination, as well as suppression of plant growth. Microscopic examination of sections of zone of maturation of the root of seedlings showed that silver sols significantly affect the conductive system of the central

axial cylinder. The number of xylem vessels in seedlings treated with silver sol at a stimulating concentration of 4.69 μg/ml was significantly higher compared to the control, which led to a more intensive growth of the root system and the whole plant. Silver nanoparticles also inhibit the growth of *F. sambucinum*. The growth inhibition zone at a maximum sol concentration of 300 μg/ml was 32.4±4.2 mm in diameter, and at 150 μg/ml it was 28.4±3.9 mm. The minimum concentration inhibiting the visible growth of the test strain *F. sambucinum* was 18.75 μg/ml (growth inhibition zone 11.7±0.8 mm). The presented data indicate the possibility of using sols of stabilized silver nanoparticles to stimulate seed germination and plant growth and to protect plants against pathogens.

Keywords: silver nanoparticles, plant growth, germinative energy, seed germination, antifungal activity, *Lepidium sativum*, *Fusarium sambucinum*

Biotechnologies and nanotechnologies in plant farming are promising due to the wide opportunities to increase crop yields by increasing plant resistance diseases, pestsn and adverse environmental factors [1]. The use of nanoparticles (NPs) with a growth-stimulating effect, antifungal, antibacterial, insecticidal activity, and prolonged release of minerals and herbicides opens up prospects for increasing crop productivity and a better storage of seeds and food products to overcome food shortages [2]. Among metals, silver nanoparticles (Ag NPs) occupy a special place due to a wide spectrum of biological activity [3, 4].

The Ag NPs are of interest as a tool to stimulate plant growth and protection [5-7]. Ag NPs, especially at high concentrations, are able to penetrate into plant tissues and accumulate [6, 8, 9]. However, NPs can have both a stimulating and an inhibitory effect on plant growth [10]. Thereof, a comprehensive understanding the NPs suitability for practical use and mechanisms of their action on a plant requires additional studies. An important area of Ag NPs application is plant protection from pathogenic fungi which significantly reduce productivity and cause contamination of animal feeds and agricultural products with hazardous mycotoxins [11]. The fungicidal properties of Ag NPs against plant fungal pathogens *Rhizoctonia solani*, *Fusarium semitectum* [12-14], *Bipolaris sorokiniana*, *Magnaporthe grisea* [15, 16], *Alternaria solani*, *Pythium spinosum*, *Pythium aphanidermatum*, *Cylindrocarcupon destructum*, *Didymella bryoniae*, *Stemphylium lycopersici*, and *Monosporascus cannonballus* [17], however, the potential of the antifungal activity of Ag NPs has not been fully dislosed.

The use of Ag NPs in agriculture necessitates reliable, efficient, and inexpensive methods for the synthesis of nanoparticles [18, 19]. Environmentally friendly reducing agents with low toxicity to humans and stabilizers of natural origin are most preferable, e.g., various polymers [20, 21] and arabinogalactan, a polysaccharide from Siberian leaf tree (*Larix sibirica*) and garden purslane (*Portulaca oleracea*) [22, 23]. Due to water solubility, thermal stability, biocompatibility, and the possibility of using it as a reducing agent and stabilizer in the synthesis of Ag NPs, arabinogalactan is recognized a promising biopolymer for the development of nanostructures and nanocomposites for agriculture [24, 25].

Reduction from silver nitrate by the so-called “green synthesis” method using fungal mycelium [26] and plant extracts [27, 28] is a safe and efficient technique to produce Ag NPs. Despite the attractiveness of the reduction method, the use of arabinogalactan seems to be more preferable, since, in addition to biosafety, biogenic synthesis occurs under more controlled conditions, and, therefore, nanoparticles have properties that are more predictable.

In this work, we have established for the first time that the pre-treatment of cress (*Lepidium sativum* L., cv. Curled) seeds with silver nanoparticles stabilized with the biopolymer arabinogalactan and dioctyl sulfosuccinate affects the germination energy, germination rate and some anatomical and morphometric parameters of seedlings. Our findings have shown for the first time an inhibitory effect of Ag NPs on the growth of the pathogenic fungus *Fusarium sambucinum*.

The aim of this work was to assess the potential of silver nanoparticles stabilized with arabinogalactan and dioctyl sulfosuccinate as a stimulant of the growth of Curled cress and an antifungal agent against mycotoxin-forming micromycete *Fusarium sambucinum* VKPM F-900.

Materials and methods. For silver nanoparticle synthesis, we used silver nitrate (JSC LenReaktiv, Russia), ammonium hydroxide (27%), sodium dioctyl sulfosuccinate Aerosol-OT, or bis(2-ethylhexyl) sulfosuccinate, sodium salt (Labtex, Russia), and arabinogalactan (Fluka, Germany). A solution of silver nitrate was added to a 0.2% solution of arabinogalactan heated to 90 °C with vigorous stirring. The reduction reaction was carried out for 40 min at the same temperature and pH ≥ 10.0, followed by the addition of sodium dioctyl sulfosuccinate to a final concentration of 0.8% and gradual cooling to room temperature. Electrokinetic potential of silver nanoparticles was determined on a Photocor compact Z analyzer (OOO Photocor, Russia). Transmission electron microscopy was performed using a LEO 912 AB microscope (Carl Zeiss, Germany) at an accelerating voltage of 100 kV. To prepare the samples, a drop of sol was applied to copper grids with a diameter of 3.05 mm, covered with a thin polymer film, and dried at room temperature. The size distribution of Ag NPs was determined by processing the obtained micrographs using ImageTool 3.00 software for the analysis of optical images (UTHSCSA, USA).

The effect of Ag NPs on germination energy, seed germination, hypocotyl and root growth of cress cv. Curled was evaluated in Petri dishes. The seeds were incubated for 1 h in sols of nanoparticles with different silver concentrations. i.e., 1.17, 2.34, 4.69, 9.38, 18.75, 37.5, 75, and 150 μg/ml (three replicates of 100 seeds per each treatment). Control seeds were incubated in water. After the end of the incubation, the seeds were germinated in Petri dishes on a wet filter paper in the dark at 20 °C. Germination energy was measured on day 3, the germination rate on day 5, the length of the hypocotyl and the main root of the seedlings on day 7.

The morphology of *L. sativum* cells was examined microscopically using root sections of 7-day-old seedlings treated with sols with stimulating and inhibiting Ag concentrations (4.69 and 18.75 μg/ml, respectively). Seedlings not treated with sol of Ag nanoparticles served as control. In the zone of seedling roots, 100-150-μm thick unfixed cross sections were prepared manually, without preliminary or subsequent fixation. The sample for each treatment consisted of 30 plants (10 from each of three independent experiments). Sections were embedded in water:glycerol (1:1) liquid and examined under a light microscope LOMO Mikmed-6 (LOMO JSC, Russia) at ×100 and ×400 magnifications. Images were obtained using a digital camera attachment Canon Digital IXUS 80 IS (Canon, Japan) and processed using the Microsoft Office Picture Manager program. The quantitative processing of photographs (n = 10 for each experimental group) was carried out using the CellProfiler program (https://cellprofiler.org) [29].

The antifungal activity of Ag nanoparticle sols was assessed by the agar diffusion method [30]. Micromycete *Fusarium sambucinum* Fuckel VKPM F-900 cultured on agar Saburo medium (PanEko, Russia) was a test culture. In wells 10 mm in diameter, 400 μl of Ag sols were added (concentrations of 9.38, 18.75, 37.5, 75, 150, and 300 μg/ml, prepared by double dilutions; control was sterile water). The experiment was carried out in three replicates, 4 wells for each dose. Petri dishes with the test strain were incubated for 5 days at 27 °C and examined for the presence of zones of growth inhibition around the wells.

The results were statistically processed using Microcal Origin 8.0 software (OriginLab Corporation, USA). All data in tables and figures are arithmetic mean values (M) and standard deviations (±SD). To identify the statistical significance of the differences, one-way analysis of variance (ANOVA) was used; differences

were considered significant at $p < 0.05$.

Results. The Ag sol was obtained by the reduction of silver nitrate using arabinogalactan, which simultaneously acted as both a reducing agent and a stabilizer for nanoparticles. To increase the stability of the Ag sol, sodium dioctyl sulfosuccinate was added. According to transmission electron microscopy data, the preparation contained spherical Ag NPs (Fig. 1, A). The average calculated diameter of the nanoparticles was 11.40±3.96 nm (see Fig. 1, B), zeta potential –24 mV.

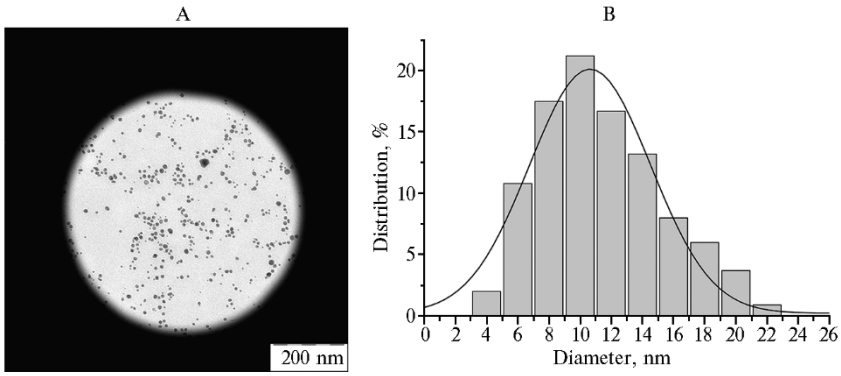

Fig. 1. Shape and size of Ag nanoparticles stabilized by arabinogalactan and Na-dioctyl sulfosuccinate: A — Ag sol (transmission electron microscopy, LEO 912 AB, Carl Zeiss, Germany; ×1500 magnification), B — distribution of nanoparticles by size (UTHSCSA ImageTool 3.00).

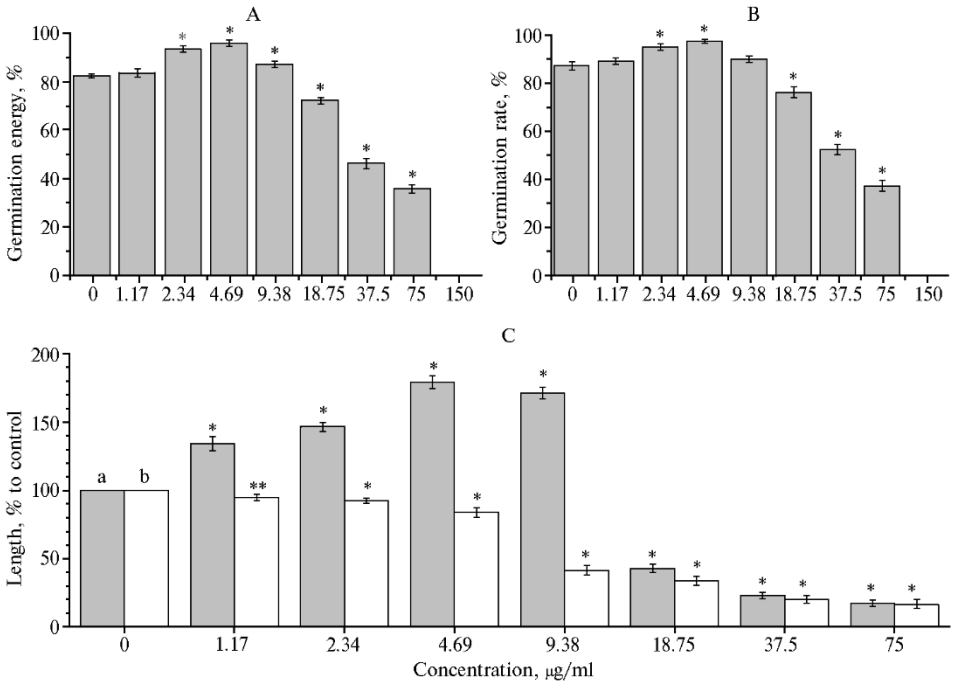

Fig. 2. Germination energy (A)**, germination rate** (B)**, the length of the main root** (a, C) **and hypocotyl of seedlings** (b, C) **in cress (*Lepidium sativum* L., cv. Curled) upon treatment with sols of nanoparticles with different concentrations of Ag** ($n = 10$, M±SD)**.**
*, ** Differences from control are statistically significant at $p < 0.01$ and $p < 0.05$, respectively.

On day 3, the germination energy in the control was 83.00±0.82% (Fig. 2, A). At the lowest Ag concentration (1.17 μg/ml), no stimulation occurred (the indicators did not differ statistically from the control). At 2.34 and 4.69 μg/ml, a pronounced stimulating effect occurred. At 4.69 μg/ml, it reached a maximum of 96.5±1.29% (13.5% higher than in the control, $p = 2.1 \times 10^{-6}$). Seeds exposed to

sols with high Ag concentrations (9.38-150 μg/ml) exhibited dose-dependent inhibitory effects. The Ag concentration of 150 μg/ml showed the maximum inhibitory effect, and the seeds did not germinate.

Seed germination rate was determined on day 5. The seeds exposed to Ag concentration of 1.17 μ`g/ml, showed no noticeable effect ($p = 0.11$) (see Fig. 2, B). At 2.34 and 4.69 μg/ml, the stimulation occurred, with an 8.8 and 11.7% increase in germination rate compared to the control ($p = 3.5\times10^{-4}$ and $p = 3.7\times10^{-5}$). The germination capacity of seeds incubated in 9.38 μg/ml Ag sol was comparable to the control ($p = 0.04$). At higher Ag concentrations, this indicator consistently decreased and was significantly lower than in the control ($p < 0.01$).

On day 7, at 1.17, 2.34, and 4.69 μg/ml Ag, the development of hypocotyl slightly slowed down while a significant dose-dependent root stimulation occurred. The length of the root significantly exceeded the control (by 34.4%, $p = 8.9\times10^{-4}$; 46.4%, $p = 8.2\times10^{-5}$ and 79.1%, $p = 6.3\times10^{-5}$). At 9.38 μg/ml Ag in sol, the length of the hypocotyl was significantly (by 58.7%, $p = 5.8\times10$ 5) less and the length of the root was 71.3% higher than in the control ($p = 5.8\times10$ 5), which indicates a significant stimulating effect of Ag NPs on root growth while suppressing hypocotyl growth. Upon incubation of seeds in sols with higher Ag concentrations (18.75, 37.5, and 75 μg/ml), the inhibitory effect consistently increased towards both hypocotyl and root ($p < 0.01$) (see Fig. 2, C).

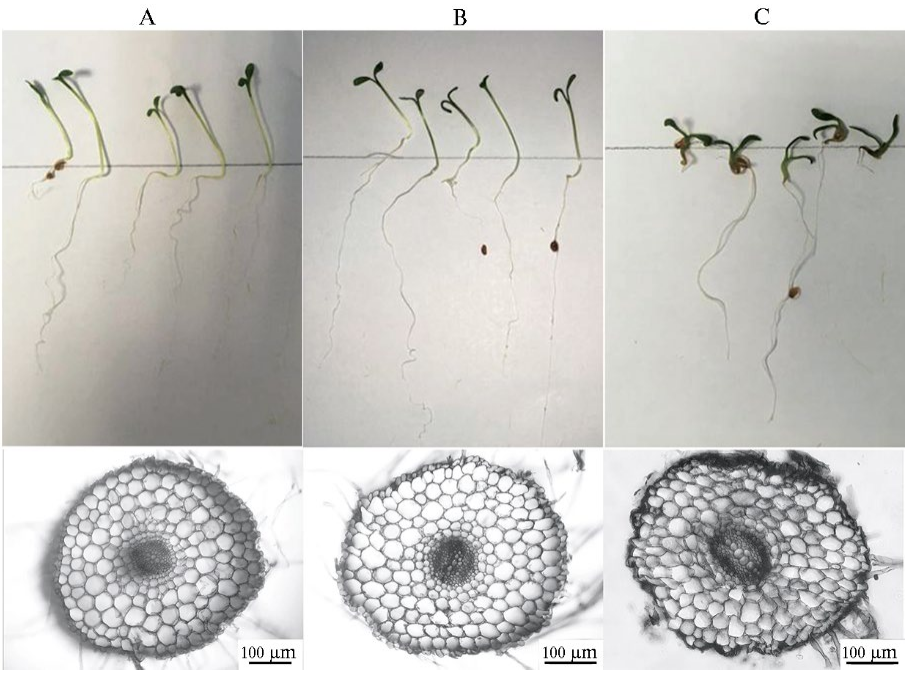

Fig. 3. Seedlings of cress (*Lepidium sativum* L., cv. Curled) 7 days after seed germination in control (A) **and upon seed exposure to Ag sols with concentrations 4.69** (B) **and 18.75 μg/ml** (C)**:** at the top — general view; below — photomicrographs of root cross sections (light microscope LOMO Mikmed-6, Russia, magnification ×400).

Root sections both in control (without seed pre-treatment) and upon seed treatment with Ag sols showed a characteristic anatomical picture of the primary root structure (Fig. 3). The integumentary tissue (a single-layer epiblema), the primary cortex, consisting of 3-4 layers of the mesoderm and one inner layer of the endoderm, and the central axial cylinder of the diarchic structure were distinguishable. The cells of the mesodermal parenchyma were round-oval in shape with more or less pronounced rectangular intercellular spaces.

Anatomic structure of roots in seedings of cress (*Lepidium sativum* L., cv. Curled) upon treatment with sols of nanoparticles with different concentrations of Ag (n = 3, M±SD)

Ag concentration, μg/ml	Number of vessels.	Diameter of parenchyma cells, μm
Control (no treatment)	12.5±3.8	58.3±12.7
4.69	21.5±5.6*	34.2±5.9*
18.75	13.9±4.1	35.6±9.5*

* Difference from the control are statistically significant at $p < 0.05$.

Comparing the root anatomical structure of the control and test seedlings, we revealed the Ag sol at a stimulating concentration of 4.69 μg/ml to provide the formation of polygonal parenchymal cells with a 41.3±12.4% decrease in size compered to control (Table). The intercellular spaces of the primary cortex parenchyma tended to decrease and practically disappeared at the inhibitory concentration (18.75 μg/ml). Inhibitory concentration also affected epiblemal cells and peripheral layers of mesoderm cells, causing thickening of cell walls and a 38.9±8.8% decrease ($p < 0.05$) in the size of parenchymal cells compared to control (see Table). Ag NPs had a significant effect on the conducting system of the central axial cylinder. The number of xylem vessels in the roots upon treatment with a stimulating Ag concentration was significantly higher ($p < 0.05$) compared to the control and an inhibitory concentration (see Table).

The size of nanoparticles directly influences not only their physicochemical properties, but also biological activity [31]. The smaller the NPs are, the higher their specific surface area and activity in overcoming physical and biological barriers. In our work, we used Ag NPs of very small size (11.40±3.96 nm) stabilized with arabinogalactan and dioctyl sulfosuccinate, which may be one of the important reasons for their active penetration through the seed coat and stimulation of germination energy, germination rate, and seedling growth. Obviously, stimulation can occur at moderately low concentrations (2.34 and 4.69 μg/ml), since higher concentrations inhibit plant growth as a result of damaging effects on biomembranes, macromolecules and disruption of the integrity of cell organelles. This conclusion is consistent with the data of Kaveh et al. [32]. The sol of high Ag NPs concentration (18.75 μg/ml), on the contrary, inhibits the formation of conductive elements in roots, suppresses root growth, and retards plant development.

The positive effect of nanoparticles on plant growth may be associated with the stimulation of photosynthetic processes, as well as with the activation of the auxin synthesis system in meristematic tissues [18]. Our study of root morphology showed that the action of 4.69 μg/ml Ag NPs led to the formation of numerous conductive elements, which was one of the important reasons for the activation of growth both roots and the entire plant due to an improved supply of water and minerals. The effect of dose-dependent stimulation of root growth that we revealed is consistent with the data of Geisler-Lee et al. [33] on accumulation of silver nanoparticles in the root cap in seedlings of *Arabidopsis thaliana*. It is obvious that the pattern of NPs distribution in a plant affects the predominant growth of certain tissues. However, the mechanism of the action has not yet been disclosed.

In this work, we also established the ability of stabilized metallic silver nanoparticles to suppress the growth of the pathogenic fungus *F. sambucinum*. Fungi of the genus *Fusarium* are mold pant pathogens that produce mycotoxins hazardous to human health [11]. Since the sources of mycotoxins can be infected green plants, animal feed, and food, an important task is to find new and expand the range of available means of combating mold fungi.

Sols had a dose-dependent inhibitory effect on the growth of *F. sambucinum* VKPM F-900. At 9.38 μg/ml concentration, we did not observe inhibition of fungal growth. The minimum concentration which inhibited the visible growth of the *F. sambucinum* test strain, was 18.75 μg/ml (inhibition zone of 11.7±0.8 mm). At 37.5, 75, 150, and 300 μg/ml, the diameters of the growth inhibition zone were 12.9±0.9, 21.1±2.7, 28.4±3.9, and 32.4±4.2 mm. The antifungal effect may be due to damage to the cell wall and phospholipid membranes by Ag nanoparticles, as well as to the disruption of the respiratory chain and the nuclear DNA of the fungus caused by silver ions dissociating from nanoparticles in biological media [34]. Inhibition of the growth of *F. sambucinum* allows us to consider the drug as a potential means of increasing yields and protecting plants and agricultural products from mold fungi and mycotoxins hazardous to human health.

Thus, in *Lepidium sativum* L. cv. Curled, the exposition of seeds to sols of nanoparticles (NPs) with a silver concentration of 2.34 and 4.69 μg/ml has a stimulating effect on the germination energy, germination rate and growth of the root of seedlings while slightly decelerates the hypocotyl growth. Ag NPs at a concentration of 4.69 μg/ml stimulate the development of the root conducting system in the seedlings. Sols with Ag concentrations exceeding 4.69 μg/ml exhibit toxic effects and inhibit plant growth. Exposure to sols of Ag NPs with a concentration of 18.75 μg/ml and higher leads to a significant decrease in the energy of germination, germination rate and suppresses plant growth. Ag NPs sols also inhibit plant pathogen *Fusarium sambucinum* (the minimum inhibitory concentration is 18.75 μg/ml). Therefore, silver sols stabilized with arabinogalactan and dioctyl sulfosuccinate have a significant potential as plant stimulants which can also protect from plant pathogens. A better understanding of advantages and obstacles of using stabilized silver sols in agriculture requires additional in-deep studies.

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