The stimulating effect of nanoparticle suspensions on seeds and seedlings of Scotch pine (Pínus sylvéstris)

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Abstract. Metal nanoparticles have unique properties that increase the growth and development of seeds of both crops and trees, thereby increasing plant productivity. The treatment of seeds with metal nanoparticles may ensure getting a high quality planting material from seeds with its further preservation. The possibility of using suspensions of nanoparticles of iron and copper in the range of concentrations from $2 \cdot 10^{-4}$ to $2 \cdot 10^{-2}$ % when preparing the planting material in nurseries and in the conditions of the forest zone on various soils is shown. It has been revealed that germination of seeds in iron and copper nanoparticles (30-60 nm) suspensions with metal concentration of $2 \cdot 10^{-3}$% results to the increase of the plant's resistance to infectious lodging (by 1.9 times), the survival rate (by 40-60%), and it's annual height (by 15%) compared to control. Although the use of nanoparticles in germination promotes the peroxidase activity, it inhibits catalase activity by showing the plant resistance to stress, while increasing growth processes. Seed germination increases to 10 % above the control, whereas the root growth increases by 25-30 %.

1. Introduction

Climate change, frequent fires and unjustified deforestation require increasing the productivity of forest stands and their quality. To solve this problem, it is necessary to constantly improve the technology of growing planting material, taking into account the specific soil and climatic conditions. The productivity of forest nurseries in Russia is very low, and the level of beneficial use of seeds of pine and spruce does not exceed 15 % [1].

Therefore, development and implementation of effective approaches to improve the sowing qualities of pine and spruce, the growth of seedlings, and increase their disease resistance [2-4] is highly important.

In order to improve sowing qualities of seeds and to enhance the germination energy, various methods are used to get their biological system out of dormancy. The techniques used to disturb the dormancy of the embryo are usually aimed at shifting the relationship between hormones in favor of growth stimulants. This may be accompanied by either a decrease in the level of endogenous inhibitors or an increase in the level of growth stimulants. A promising method of preparing seeds of conifers is their pretreatment with metal nanoparticles (NPs) [5]. The seed dormancy is interrupted and the biological system goes into an excited state. Enzymes are activated in the endosperm and in the

embryo of the seeds, leading to an enhanced transfer of the spare nutrients from the unabsorbed for the embryo and the seedling form to an accessible one in the form of sugars, free amino acids and organic acids. Oxidative processes, leading to the formation of physiologically active substances that positively affect the embryo and cause its intensive growth, increase. Breathing increases and physical and chemical changes occur in a seed, which ensures the beginning of the growth of the embryo.

The small size of metals (no more than 100 nm) allows them to pass through biological membranes, accumulate in the internal environment, possibly integrate into DNA or proteins and, thus, change their functions. A large specific surface area of metals in a nanoscale state increases the chemical potential at interphase boundaries and leads to an anomalous increase in solubility and reactivity. These distinctive properties can lead to the manifestation of various effects on living systems, including plants. Both positive [6,7] and negative effects on plant growth have been identified [8,9]. Technologies, created with the help of NPs, can be aimed at increasing yields and creating inductors of stress tolerance of agricultural plants to adverse environmental factors, etc. [10-13].

A method of disposable treatment of plant seeds before planting with nanoparticles based on biogenic metals: iron, copper, which increase seed germination energy, stimulate plant growth and development, increase crop yields, change the amount of biopolymers for each crop, depending on their genetic directionality. The peculiarity of the chemical interaction of NPs with a liquid medium is one of the determining factors in stimulating the development of plants, which makes it possible to use them as micronutrients and growth stimulants that have a prolonged effect, which is reflected in their prolonged influence on the regulation of mineral nutrition, carbohydrate metabolism, photosynthesis and cell respiration [14-16].

Therefore, the aim of the work was to investigate the treatment of seeds of Scotch pine (Pínus sylvéstris) with iron and copper NPs to obtain an environmentally sustainable product with desired properties.

2. Experimental Part

2.1. Experimental setting

Employees of the Faculty of Forestry, Agrochemistry and Ecology together with the Centre for Nanotechnologies and Nanomaterials of Ryazan State Agrotechnological University, Russia have had researches having the Scotch pine as the object since 2010. Seeds of Scotch pine (second class) were taken from the cone-drier of Ryazan region. We used nanoparticles of iron (Fe NPs) and copper (Cu NPs) purchased from Producer, Russia. The seeds were exposed to NPs suspensions containing $2 \cdot 10^{-4}$ %, $2 \cdot 10^{-3}$ %, $5 \cdot 10^{-3}$ %, $2 \cdot 10^{-2}$ % and 10 % of iron and copper.

After exposure, we determined: germination energy and laboratory germination according to the method [17] at 0 ... 60 ° C ± 1 ° C, in compliance with GOST 13056.6-97 [18], morphological and physiological parameters of germination and seedling development according to [17,19]. Estimation and registration of sprouted seeds when determining the germinating energy and viability were examined after 7-day exposure. Weighing was carried out with the help of analytical balance GN - 202 (Producer, Japang).

Seeds were germinated on a gel-like cultivation medium in Petri dishes. The gel forming agent is a polysaccharide derived from seaweed (Difco agar). Parameters of microbiological agar are as it follows: sulfates ≤ 1 %; calcium ≤ 0.4 %; magnesium ≤ 0.2 %; total nitrogen ≤ 0.25 %; pour point ≥ 36 ° C; melting point of 1.2 % gel ≤ 5 ° C; pH (1.2 % gel) varies from ≤ 6.1 to ≥5.7 after autoclaving. The seeds intended for germination were pre-heated at a temperature of 30-40 ° C for 7 days in a thermostat.

The field experiment started in the spring of 2010 at "Solotchinskoe forest division", Ryazan region, Russia. The soil was sod-podzolic sandy (fresh coniferous forests). The total area of the experiment was 11.6 hectares: 6.2 hectares without processing and 5.4 hectares of arable land. Two linear sample plots were used for registration.

For the results validity, the experiment was also carried out on the experimental field of the agrotechnological experimental station of Ryazan State Agrotechnological University, Russia. The soils of the experiment were gray forest, heavy-loamy. The replication was 3-fold.

May and summer of 2010 in Moscow and Ryazan regions of Russia were abnormally hot. The average monthly temperature was 26.1 °C. In 2012, plots with an area of 50m^2 were planted with 100 standard seedlings of Scotch pine, the seeds of which were treated with a suspension of nanoparticles. The recording method was continuous. The plant spacing was 1.0 m and the row spacing was 2.0 m.

Since 2013, field experiments have been taking place at "Pervomayskoe forest division", Ryazan region, Russia. The soil is sod-podzolic sandy (fresh coniferous forests). The total area of the experiment was 6.6 hectares: 4.2 hectares without processing and 2.4 hectares of arable land. Some linear sample plots were used for registration.

Since in laboratory experiments it was shown that Fe NPs stimulate seed growth more actively than copper, in field trials, seed soaking in an aqueous suspension with an optimal iron concentration of $2 \cdot 10^{-3}$ % was used as experimental options for 20 minutes. In addition, for comparison, the soaking of seeds was carried out in solutions of widely used growth regulators [20,21]: Agat-25K with exposure of 20 minutes and a concentration of 0.015 % (according to the manufacturer's recommendations) and Zircon P, with exposure of 18 hours and a concentration of 0.5 g / kg of seeds (as recommended by the manufacturer). The control seeds were soaked in water for 20 minutes.

2.2. Defining biochemical parameters of seedlings

Determining the activity of catalase, peroxidase and superoxide dismutase in plant tissues took place at the faculty of general chemistry with the course of bioorganic and organic chemistry, microbiology and biochemistry of Ryazan State Medical University, Russia. The activity of soluble peroxidases and catalase was determined by the photometric method, which successfully proved itself in numerous studies on various biological objects [22-25] using SF-2000 spectrophotometer (OKB SPECTR CJSC, Russia).

Test charges of plant samples weighing 800-900 mg were mechanically crushed and ground in a porcelain mortar with a porcelain pestle with the addition of 10 ml of cold phosphate buffer 1/15 M pH 6.7. The resulting suspension was completely transferred to a conical centrifuge tube and the water-soluble components were extracted at 4 ± 2 °C for 1 hour with 3-fold resuspension. After the time of extraction, the samples were centrifuged at 3000 rpm for 15 minutes. The resulting supernatants were immediately separated from the pellet and transferred to Eppendorf micro centrifuge tubes. Both native and stored at −20 ± 1 °C supernatants can be used for analysis.

2.2.1. Determining peroxidase activity in plant tissues.

To evaluate the oxidation of guaiacol in the presence of hydrogen peroxide, reagents were sequentially added to the control and test samples according to Table 1, after which the reaction was started separately in each tube with the addition of 0.5 cm^3 of 0.33 % hydrogen peroxide solution.

Then the stopwatch was turned on and the optical density of the solution was measured at a wavelength of 440 nm, immediately after application and every 30 seconds, for 180 seconds against distilled water.

Table 1. Conditions for the use of reagents when determining peroxidase activity in plant tissues.

Reagent	Control sample	Experimental sample
1/15 M phosphate buffer pH 6.7	0.5 cm^3	0.5 cm^3
Distilled water	0.5 cm^3	–
Supernatant of plant material homogenate	–	0.5 cm^3
0.05 % alcohol solution of guaiacol	0.5 cm^3	0.5 cm^3

On the linear section of each graph, calculate the rate of change of the optical density of E_{440} per 1 second was calculated: ΔE_{440} (unit opt. dens.) / T (second).

Peroxidase activity (A_{PO}) in the test material (in units of opt. dens. / g of wet tissue • second) was calculated according to the formula:

$$A_{PO} = \Delta D_{440} \bullet N/m \bullet d, \quad (1)$$

where D_{440} – the difference in the rate of increase in optical density (unit opt. dens. / second) for experimental and control samples; N – material dilution; m – the mass of test charges of the plant material; d – the thickness of the absorbing layer (mm).

2.2.2. Determining superoxide dismutase activity in plant tissues

The reaction mixtures were prepared in cylindrical glass tubes according to Table 2

To evaluate the superoxide dismutase activity, 0.1 cm^3 of a 0.5 mM quercetin solution was added to the experimental sample, a stopwatch was turned on, and the optical density of the solution was measured at a wavelength of 406 nm immediately after application and every 60 seconds for 10 minutes against the blank sample. When calculating superoxide dismutase activity, the dependency graphs of the optical density of A_{406} on time (t) for the control and experimental samples. On the linear section of each graph, ΔE_{406} was calculated for 1 minute:

$$\Delta E_{406} = (A_{406}1 - A_{406}2) / t \text{ (min)}, \quad (2)$$

Superoxide dismutase activity (A_{SOD}) was calculated in the test material (standard units / g of raw tissue):

$$A_{SOD} = ([\Delta E_{406} \text{ control} / \Delta E_{406} \text{ experimental}] - 1) \bullet N / m \quad (3)$$

where N – material dilution; m – the weight of a test charge of the plant material (g).

Table 2. Conditions for the use of reagents when determining superoxide dismutase activity.

Reagent	Blank sample	Control sample	Experimental sample
Distilled water	2.0 cm^3	1.9 cm^3	1.8 (1.85) cm^3 *
Working mixture 1 Glycine-alkaline buffer pH 10.0	0.5 cm^3	0.5 cm^3	0.5 cm^3
Supernatant of the plant material homogenate	0.5 cm^3 –	0.5 cm^3 –	0.5 cm^3 0.1 (0.05) cm^3 *

Note: * the amount of the introduced component depends on the activity of superoxide dismutase in the analyzed material. The amount of supernatant applied was selected for each type of plant material so that the degree of inhibition of autoxidation of quercetin in the experimental sample was 2-2.5 : 1 relative to the control. The volume of water changed in accordance with the change in the volume of the homogenate so that the volume of the reaction mixture was 3.0 cm^3.

2.2.3 Determining catalase activity

A test charge of the plant tissue weighing 250 mg was ground in a cooled mortar in 0.5 ml of extraction buffer. The homogenate was centrifuged for 5 minutes at 12,000 g. Samples (supernatant) were stored in a refrigerator (4 °C).

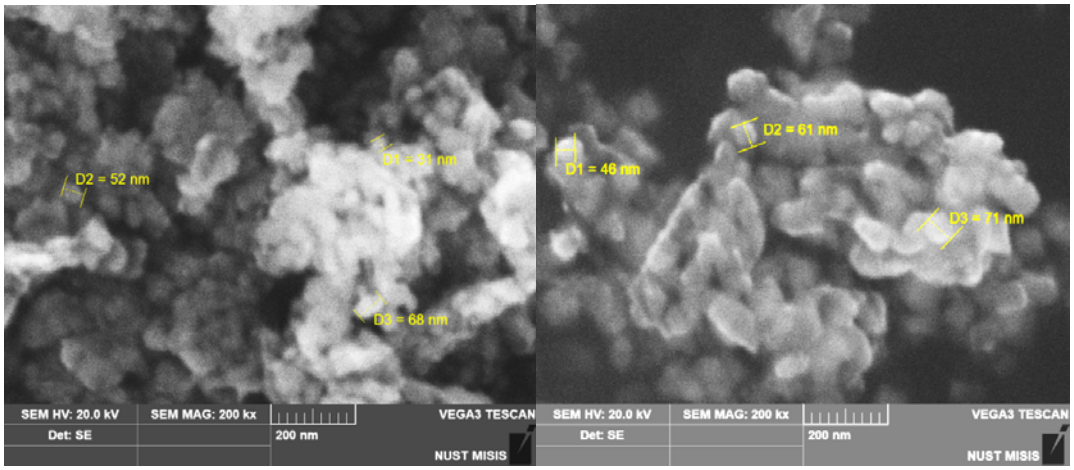
The reaction mixture contained 2.95 ml of 50 mM K, Na – phosphate buffer (pH 7.0) and 30 µl of the supernatant. The reaction was started by adding 20 µl of 0.6 M hydrogen peroxide to the reaction mixture. The control cuvette contained the same reagents, but hydrogen peroxide was not added. Catalase activity was determined by the change in optical density at a wavelength of 240 nm every second for 120 s. The calculation of catalase activity in relative units per gram of dry weight was carried out according to the formula:

$$A = (\Delta D \cdot V \cdot X) / (T \cdot L \cdot m \cdot \Delta m), \quad (4)$$

where A – the activity of the enzyme, ΔD – the change in optical density (subtraction of the optical density at the final point in time from the optical density at the beginning of the reaction), V – the total volume of the obtained extract, ml, X – the final dilution of the extract in the cuvette (the volume of the reaction mixture was divided by the amount of the applied extract), T – reaction time, s, L – the layer thickness, cm, m – the mass of the test charge, g, Δm – the ratio of dry weight to raw.

2.3. Characteristics of Fe NPs and Cu NPs

NPs of copper and iron 35–60 nm in size (figures 1a and 1b) were obtained at Research and Technology University "Moscow Technological Institute of Steel and Alloys", Russia by chemical precipitation of metal hydroxides from salt solutions followed by low-temperature reduction in hydrogen and passivation [26, 27]. Figure 1 shows micrographs of metal NPs.



a) b)

Figure 1. SEM images of iron (a) and copper (b) nanoparticles.

The phase composition of Cu NPs (Cu 85 %), CuO (15 %), Fe NPs (Fe 6 %) and Fe_3O_4 (94 %) was determined using X-ray phase analysis by the powder method with the help of XRD-7000 diffractometer (Shimadzu). According to thermogravimetric data, the residual oxygen content when reduced to 800 ° C was 11.6 % for Cu NPs and 12.6 % for Fe NPs.

The specific surface of the obtained copper NPs (6.5 m^2 / g) and iron NPs (42.7 m^2 / g) was measured by the method of BET low-temperature nitrogen adsorption, using analyzer "Quantachrome NOVA 1200e".

The suspension of iron and copper NPs was obtained by dispersing a test charge of particles in distilled water for 7 minutes with power of 300 W and frequency of 23 kHz using Hielsher UIP1000hd ultrasonic disperser (Hielscher Ultrasonics, Germany). The calculated amount of the suspension was introduced either into the polysaccharide nutritional mixture, or the seeds were soaked with the introductory suspension before sowing. In the control, where growth stimulants and Fe NPs were not used, the seeds were soaked in distilled water. After soaking, the seeds were dried to the state of free-running. To perform the experiment, analytical scales of the updated series VL and VL-V were used.

3. Results and discussion

3.1. Laboratory studies

3.1.1. Laboratory morpho-physiological studies. Indexes of laboratory germinating energy and viability of seeds sprouted on a gel-like cultivation medium containing Fe NPs and Cu NPs of different concentrations are presented in Table 3.

Table 3. Germinating energy and viability of the Scotch pine seeds treated with nanoparticles suspension.

Variant	Germinating energy, %	Viability, %	Variant	Germinating energy, %	Viability, %
Control	81.0±0.7	92.0±0.9	Control	81.0±0.7	92.0±0.9
Fe $2\cdot10^{-4}$	83.0±1.2	94.0±0.8	Cu $2\cdot10^{-4}$	*85.0±0.8	*96.1±1.6
Fe $5\cdot10^{-4}$	83.8±1.3	94.9±0.6	Cu $5\cdot10^{-4}$	89.1±0.6	95.9±0.8
Fe $2\cdot10^{-3}$	84.4±0.9	99.9±0.7	Cu $2\cdot10^{-3}$	84.4±0.7	96.6±0.7
Fe $5\cdot10^{-3}$	83.4±1.2	96.6±1.2	Cu $5\cdot10^{-3}$	88.0±1.1	98.0±2.0
Fe $2\cdot10^{-2}$	82.4±1.6	95.6±0.9	Cu $2\cdot10^{-2}$	85.0±0.7	94.0±1.5
Fe 10	75.4±1.4	85.4±1.1	Cu 10	80.0±1.5	91.0±2.1

Note: *-$P \leq 0.05$

Fe NPs and Cu NPs in the concentration range of $2\cdot10^{-4}$ - $2\cdot10^{-2}$ % resulted higher values of germinating energy and viability relative to the control. Viability and germinating energy when interacting with Fe NPs and Cu NPs decreased insignificantly as compared with the control only at the concentration of NPs equal to $2\cdot10^{-2}$ %. The 10 % NPs concentration had some inhibitory effect on seeds (Table 1).

Analysis of the vital indices showed that small concentrations of Fe NPs (from $2\cdot10^{-4}$ % to $5\cdot10^{-3}$ %) contributed to a significant increase in the length of the vegetative part of the seedling up to 46.9 % above the control and higher concentrations of NPs (from $2\cdot10^{-2}$ %) resulted only in 24.2 % increase. The length of the root part of the pine sprout influenced by Fe NPs exceeded the control at all concentrations, maximum up to 70.0 %. The metric indices of sprouts influenced by Cu NPs suspension resulted in a negligible increase in the length of the vegetative part (8.9 %) and a larger increase of the root part (up to 30.4 %). Cu NPs inhibited the growth of seedlings starting at a concentration of 10 %.

Thus, Fe NPs showed a larger effect on pine seeds than Cu NPs.

Weight values of Scotch pine seedlings influenced by suspensions of Fe NPs and Cu NPs are presented in Table 4. The mass of seven-day sprouts under the influence of Fe NPs exceeded the control in all variants of the experiment. The maximum increase for the vegetative part was up to 80.4 % (Fe $2\cdot10^{-3}$ %) and for the root part up to 65.0 %. The introduction of various concentrations of Cu NPs into the cultivation medium promoted some increase in the weight of the vegetative part of the seedling in all variants by 12.5-32.6 %. The weight of the root part of the sprout in a case of NPs (up to $2\cdot10^{-3}$ %) in the growing medium exceeded the control by 60 % and when the concentration was increased to 10 %, the weight decreased significantly to 52.0 % below the control.

Table 4. Weight values of 7-day seedlings of the Scotch pine exposed to nanoparticles suspension.

Variant, concentration, %	Weight of the vegetative part, g	Weight of the root part, g	Variant	Weight of the vegetative part, g	Weight of the root part, g
Control	1.84±0.21	0.40±0.02	Control	1.84±0.21	0.40±0.02
Fe $2\cdot10^{-4}$	2.39±0.80	0.47±0.01	Cu $2\cdot10^{-4}$	2.44±0.11	0.45±0.03
Fe $5\cdot10^{-4}$	2.82±0.20	0.55±0.01	Cu $5\cdot10^{-4}$	2.28±0.14	0.44±0.04
Fe $2\cdot10^{-3}$	3.32±0.32	0.66±0.02	Cu $2\cdot10^{-3}$	2.16±0.08	0.41±0.04
Fe $5\cdot10^{-3}$	3.12±0.61	0.61±0.01	Cu $5\cdot10^{-3}$	2.07±0.12	0.45±0.03
Fe $2\cdot10^{-2}$	*3.12±0.34	0.46±0.02	Cu $2\cdot10^{-2}$	2.57±0.41	0.33±0.06
Fe 10	2.46±0.35	0.29±0.02	Cu 10	1.79±0.61	0.27±0.08

Note: *-$P \leq 0.05$

Thus, it is possible to consider the maximum allowable concentration of $2 \cdot 10^{-2}$% and the optimal concentration of $2 \cdot 10^{-3}$ % both for Fe NPs and Cu NPs. However, Fe NPs are more active for Scotch pine seeds and in further studies a suspension of Fe NPs was used.

3.1.2. Biological studies

One of the most important criteria of adaptive resistance of the plant is its biochemical status. Fluctuations in the concentration of the most important antioxidant enzymes, as well as phytohormones, can reflect the negative effect caused by environmental factors on the homeostasis of the plant, i.e. some deviation of biochemical status parameters from the norm can be a sign of some toxic effect [28-30]. Some artificial nanoparticles, able to enter the plant habitat, can be considered as one of such factors.

A study of the toxicity of NPs was carried out on seeds of the Scotch pine using Fe NPs in different concentrations. The results of the experiments are presented in Tables 4 and 5.

Table 5. The activity of peroxidase (unit of opt. dens. / g of raw tissue * sec) and superoxide dismutase (conventional activity units/g of raw tissue) in roots and sprouts Scotch pine under the influence of Fe NPs

Variant, concentration, %	Peroxidase				Supeoxide dismutase			
	Root part of the seedling		Vegetative part of the seedling		Root part of the seedling		Vegetative part of the seedling	
	Absolute value	% to control	Absolute value	% to control	Absolute value	% to control	Absolute value	% to control
Control	4.63	-	6.83	-	98.04	-	159.01	-
Fe $2 \cdot 10^{-4}$	5.23	+12.96	7.60	+11.27	100.87	+2.89	168.21	+5.79
Fe $2 \cdot 10^{-3}$	5.52	+19.22	7.34	+7.47	102.36	+4.41	165.19	+3.89
Fe $5 \cdot 10^{-3}$	5.64	+21.81	8.06	+18.00	113.30	+5.57	181.43	+14.10
Fe $2 \cdot 10^{-2}$	5.02	+30.02	8.14	+19.18	126.36	+28.89	195.19	+22.75
Fe 10	6.24	+34.77	9.06	+32.65	133.30	+35.96	211.43	+32.97

Note: *-P ≤ 0.05

The content of peroxidase (Table 5) in the roots of the experimental samples of the pine grew. As the concentration of Fe NPs increases, the peroxidase activity increases by 12.96 %, 19.22%, 21.81%, 30.02 % and 34.77 % correspondingly to the control. The peroxidase activity in the vegetative part of the Scotch pine seedling also increases from 11.27 % to 32.65 % (10%) with an increase in the content of Fe NPs in the nutrient medium throughout the concentration range. Thus, peroxidase activity reacts equally to Fe NPs in the nutrient medium, increasing both in the vegetative part of the seedling and in the root, but it differs in value.

The activity of superoxide dismutase in experimental samples in the vegetative and root parts of pine seedlings also increases. When using suspensions of Fe NPs the activity of the enzyme in the roots increased slightly by 2.89 % ($2 \cdot 10^{-4}$), 4.41 % ($2 \cdot 10^{-3}$), 5.57 % ($5 \cdot 10^{-3}$), 28.89 % ($2 \cdot 10^{-2}$) and 35.96 % (10 %). The activity of superoxide dismutase in pine sprouts increases with an increase in the content of Fe NPs by 32.97 % at most (10 % concentration) as compared to the control (Table 5).

The oxidative regime in the plant regulates enzyme catalase. Its intensity is considered as the intensity and productivity of the total exchange. Catalase also protects living organisms from damage by hydrogen peroxide, formed as a result of oxidation-reduction reactions. Catalase activity (in relative units per gram of dry weight) in roots of Scotch pine seedlings being effected by Fe NPs increases, but with some increase of iron concentration the activity of the enzyme declines relative the control by 19.14 % ($2 \cdot 10^{-3}$ %) and by 2.39 % ($5 \cdot 10^{-3}$ %), but at concentrations of 10 % it races up by 34.72 % relative the control.

The enzyme activity in the vegetative part of Scotch pine seedlings decreases relative the control with an increase of iron concentration by 9.79 % when Fe $2 \cdot 10^{-3}$, by 17.04 % when Fe $2 \cdot 10^{-2}$ and only by 5.7 % when 10 % Fe.

An alternative oxidase system of cells: peroxidase and catalase, by the activity of which one can judge the viability of plants, can serve an indicator of resistance. Peroxidase and catalase compete for the substrate, hydrogen peroxide, and the activity of each enzyme depends on the work of the "partner". As a rule, the activity of catalase decreases with some increase in the activity of peroxidase.

Consequently, the use of the investigated Fe NPs promotes some increase in peroxidase activity and some decrease in catalase activity, as indicators of an increase in resistance, and growth processes are enhanced at the seedling stage. The results of the experiment prove that concentrations of Fe NPs up to $2 \cdot 10^{-3}$ % stimulate seed germination and the development of seedlings. However, at concentrations of 10 % or more, changes in the activities of enzymes are above 30%, therefore, these concentrations can be dangerous.

3.2. Field tests

The effect of the suspension of Fe NPs on the seed germination and on the biometric parameters of pine seedlings (Pínus sylvéstris) was studied in field experiments at "Solotchinskoe forest division", Ryazan region, Russia and the field of the experimental station of Ryazan State Agrotechnological University, Russia. The use of growth stimulants (Agat-25K and Zircon, P) did not exceed the best figure with the use of Fe NPs.

When seeds were soaked in a suspension of Fe NPs, uniform and rapid germination Seed germination and the growth of seedlings in natural conditions largely depend on their physical properties, temperature, availability of water and minerals. The soil is a good medium for sowing seeds due to its high water filtration capacity, good aeration and close contact between soil particles and seeds. In the process of investigations, it was determined that the optimal time for soaking seeds in suspension of Fe NPs was 20 minutes, which increased the germination energy of the control variant from 56.2 + 2.1 % to 61.9 + 2.2 % (table 6). Exceeding the 20-minute exposure significantly reduced this figure. For comparison, the seeds before sowing were soaked in widely used growth regulators Agat-25K and Zircon, P.

Table 6 - The effect of Fe NPs on seeds germination and on the biometric parameters of pine seedlings (Pínus sylvéstris).

Variant concentration, %	Germination			Aerial part			Main root		
	Field, %	*, %	**, %	Length, mm	*, %	**, %	Length of, mm	*, %	**, %
Control	56.2 ±2.6	6.8	2.8	40.4 ±3.3	14.1	5.3	173.5 ±5.2	18.3	6.5
Fe $2 \cdot 10^{-3}$ %	61.9 ±2.2	7.1	3.9	47.8 ±1.0	15.7	6.1	211.8 ±5.2	18.3	6.5
Agat-25K	58.2 ±3.9	5.8	3.4	42.2 ±1.1	14.7	5.7	189.9 ±4.1	22.1	6.9
Zircon, P	57.7 +3.2	6.9	4.0	44.0 ±0.9	15.1	6.2	186.7 ±8.77	18.4	6.7

Note: * - Coefficient of variation, ** - Test accuracy.

When soaking seeds in the suspension of iron nanopowder the uniform and rapid germination of seeds was observed, which is a necessary condition for obtaining the high-quality planting material, since this reduces the risk of seed damage by insects, fungi or other adverse conditions. So the length of the aerial part (Table 4) of the control variant was 40.4 mm. Twenty-minute soaking contributed to

the growth of the aerial part to 47.8 mm (118 % of the control). The use of growth stimulants (Agat-25K and Zircon, P) did not exceed the best figure with the use of Fe NPs.

The length of the main root (table 4) of the control variant was 173.5 mm. The use of Fe NPs increased this parameter by 22 %. The use of growth stimulants Agat-25K and Zircon, P slightly increased the length of the main root relative to the control up to 9%.

The results of the experiment prove that Fe NP concentration of up to $2 \cdot 10^{-3}$ % stimulate germination of seeds and seedlings development.

According to the results of the inventory, the survival ability of Scotch pine seedlings at "Solotchinskoe forest division" was 68 % in the control (without treatment) and 76 % in the considered variant, i.e. the increase in survival ability was 8 % and their preservation in the control (without treatment) was 96 % and that in the studied variant was 100 %, so the increase was 4 %. The pine planting material had the following biometric parameters: the average height of plants was 4.81 $\pm$ 0.05 cm (accuracy 4.58 %) and the average stipitate diameter was 1.34 $\pm$ 0.03 mm. The use of Fe NPs contributed to an increase in the parameters of linear growth, both in comparison with the initial data, and in comparison with the control variant. The following data were obtained: the average height of plants in the control increased to 168.24 $\pm$ 7.2 cm and the use of Fe NPs increased the average height of plants to 243.65 $\pm$ 7.94 cm, the average diameter of the stipitate in the control increased to 84.53 $\pm$ 5.67 mm and the use of Fe NPs increased this index to 92.31 $\pm$ 6.19 mm.

The survival ability of Scotch pine seedlings in the field of the agrotechnological experimental station at Ryazan State Agrotechnological University, Russia was 20 % in the control (without treatment) and 68 % in the studied variant. One of the research tasks was to study the influence of Fe NPs on the resistance of pine seedlings to diseases. During the first year of growing the death of seedlings in the control took place from infectious lodging, root-collar scorch, sunburn, drowning, squeezing and drought, and it ranged from 12.4 to 19.8 %. The share of seedlings loss from lodging was about 50 % of the total loss. Preservation in the experimental variants was 96 %. The effect of treatment on resistance to infectious lodging both in variants with additional pretreatment and without it was practically the same: the pine resistance increased in most cases in 1.6-1.8 times.

In the second and third years of growing, the loss of the control seedlings of the pine was due to squeezing, drought, spring frosts, losses during manual and mechanized care, and also a backlog in growth. In different years the death of pine seedlings was in the range from 16.1 to 23.7 %. However, when treating with Fe NPs, the difference with the control in terms of the loss magnitude was reliable and 80 % lower.

When planting, the seedlings of the pine had the following biometric parameters: the average height of the plants was 4.31 $\pm$ 0.06 cm and the average diameter of the stipitate was 1.21 $\pm$ 0.04 mm. During seven years of the experiment, the linear parameters of plants changed significantly. The average height of plants in the control increased to 204.71 $\pm$ 10.78 cm and the average diameter of the stipitate to 85.27 $\pm$ 11.18 mm. The use of Fe NPs increased the average height of plants to 255.67 $\pm$ 11.22 cm and the average diameter of the stipitate to 94.52 $\pm$ 10.31 mm.

The survival ability of Scotch pine seedlings at "Pervomayskoe forest division" was 70 % in the control (without treatment) and 98 % in the studied variant, i.e. the increase in survival ability was 28 % and preservation of Scotch pine seedlings in both variants was 100 %.

As a result of applying Fe NPs, the height of the plants increased by 15 % annually with a commensurate increase in the diameter of the stipitate in the zone of the cingulum and the average root length by 69 %. This indicates that the use of Fe NPs is important in creating pine forest populations.

4. Conclusion

Treatment of pine seeds with suspensions of Fe NPs and Cu NPs in laboratory setting improved their seeding qualities and reduced the probability of seedlings' mold damage considerably. The presence of Fe NPs and Cu NPs sized 35-60 nm in a nutrient medium when germinating Scotch pine seeds in a concentration range of $2 \cdot 10^{-4}$ % - $2 \cdot 10^{-2}$ % contributed to a significant increase in germinating energy and viability. The higher content of NPs (10 %) causes availability and germinating energy decrease,

hence high concentrations inhibit availability of Scotch pine seeds. Changes in the activity of enzymes for the presence of NPs do not exceed a deviation of more than 30 %, therefore, they have some stimulating effect. At concentrations of 10 %, deviations are more than 40 %, indicating the danger of such concentrations. Based on the results of the experiment, the concentration of up to $2 \cdot 10^{-2}$ % can be considered the maximum allowable concentration both for Fe NPs and Cu NPs and the concentration of $2 \cdot 10^{-3}$ % can be considered as the optimal one for both NPs. The variants with Fe NPs and Cu NPs had a more powerful root system, than in the control, which allows improving the growth of the aerial part. However, Fe NPs are more active for Scotch pine seeds and their use is more efficient.

One of the main types of work that determines the maximum yield of the high-quality planting material is the preparation of seeds for sowing. To prepare the seeds of the Scotch pine for planting we have proposed suspensions of Fe NPs and Cu NPs, which at the same time serve as a source of microelements and growth stimulants, which have a prolonged action in small quantities. This method finds practical application in the sowing departments of temporary and permanent forest nurseries for growing the Scotch pine. It is simple to perform, does not require any significant additional costs and special equipment, and fits well with the technology of growing the pine planting material.

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