Peculiarities of growth and development of *Pinus sylvestris* L. when cultivated in the medium containing copper nanoparticles

Z N Ryabinina¹, R G Kalyakina², O N Nemereshina³, N F Gusev², M V Ryabukhina¹

¹Federal Research Centre of Biological Systems and Agro-Technologies of the Russian Academy of Sciences, 29, 9 Yanvarya str., Orenburg 460000, Russia
²Orenburg State Agrarian University, 18, Chelyuskintsev str., Orenburg, 460000, Russia
³Orenburg State Medical University, 6, Sovetskaya str., Orenburg, 460000, Russia
E-mail: kalyakina_railya@mail.ru

Abstract. At present, ecologists, physiologists and other scientific researchers are facing the problem of little knowledge of the action of nanomaterials, which makes it impossible to assess the potential risks of using nanomaterials. The widespread use of copper nanoparticles, in particular the use in the presowing treatment of plant seeds, makes it relevant to study the characteristics of plant growth when copper is introduced into the medium. In the present work, the growth features of *Pinus sylvestris* L. — Scots pine, were studied during cultivation in a medium containing copper nanoparticles. Research has shown that the cultivation of *P. sylvestris* L. in a medium containing copper nanoparticles at concentrations of 0.025–0.1 M completely blocks the development of the root system. Cultivation in medium containing copper nanoparticles at a concentration of 6.25 mmol reduces the length of the root to 27.9 ± 3.8 mm, and also reduces the mass to 19.6 ± 1.9 mg.

1. Introduction
Science has accumulated a significant knowledge base about the features of plant adaptation to environmental conditions with the content of nanomaterials. The first works on this topic falls on the 19th century and are associated with studies of the effect of carbon black on plants in the UK as industrial production develops. Technogenic change of edaphic conditions and, accordingly, processes of mineral nutrition of plants is considered as one of the leading causes of forest degradation in the vicinity of industrial enterprises. However, as practice shows, the effect of nanometals has positive aspects, as with strict dosing, nanometals have a stimulating effect on the development of higher plants, both agricultural and wild [1-4]. Obviously, the unique properties of nanomaterials — penetrating ability across biological membranes, a significant specific surface area, reactivity in the near future will ensure the dominance of nanoforms in the market of micronutrients and components for crop production. Currently, preparations based on nanometals, in particular copper, are used in seed dressing and plant growth stimulation [1, 5-7]. Presowing treatment of seeds with Cu, Zn nanopowders has a positive effect on wheat, rapeseed and maize. However, copper in excess has a toxic effect, and the root barrier plays...
a significant role in the formation of tolerance to an excess of this metal [8-11]. The effect of nanopowders on the growth and development of woody plants has not yet been practically studied.

2. Materials and methods
The object of the research was the first-class seeds of *P. sylvestris* L., collected on the territory of the Orenburg region and having undergone preliminary stratification [12]. The experiments were carried out using copper nanoparticles (103 ± 2 nm coated with a thin oxide film of CuO), copper microparticles (size 40 μm, purity 99.5%), and also copper sulfate [13]. Nanoparticles were obtained at the Institute of Energy Problems of Chemical Physics, Russian Academy of Sciences (Moscow) on the MiGen facility using the method of high-temperature condensation. Copper microparticle manufacturer - Sigma - ALDRICH Chemie Gmbh, Riedstr (Germany). At the first stage of the study, weighed the test substances (100 mmol / l of each substance) were diluted with distilled water (10 ml) and dispersed by sonication at a frequency of 35 kHz in a «Sapphire TTC» bath type source 30 minutes, after which fourfold dilutions were prepared. The scheme of the experiment is presented in table 1.

| Copper Forms          | Amount of substance |
|-----------------------|---------------------|
|                       | 100                 |
|                       | 25                  |
|                       | 6.25                |
|                       | 1.56                |
|                       | 0.39                |
| Copper sulphate       | 4.1                 |
| Copper microparticles | 5.1                 |
| Copper nanoparticles  | 6.1                 |

Quartz sand pretreated was used as a substrate for germination. Biotesting was performed in accordance with GOST 15150 in laboratory conditions [14]. The experiment was carried out according to the following scheme: pretreated substrate was placed in containers, then *P. sylvestris* L. seeds were deepened and irrigation was performed with pre-prepared suspensions or solutions. Irrigation of the control samples was carried out with distilled water. After that, the containers were placed in a thermostat with an optimal germination regime for a period of 14 days, if necessary, the substrate was moistened with the test solutions. Seed germination and morphological features of seedlings served as diagnostic signs [15].

For transmission microscopy, samples of the *Pinus sylvestris* L. root system (apical part, root cap, primary meristem) were fixed in 2% glutaraldehyde prepared in phosphate buffer, followed by refixation in 1–2% OsO4 solution. After dehydration in ethanol solutions of upward concentration, the samples were contrasted with uranacetate and enclosed in epon. Ultrathin sections were obtained on a Tesla microtome, stained with uranyl acetate and lead citrate according to Reinalds. Microscopy was performed using a JEM- & A electron microscope (Japan).

3. The study the effect of nanoparticles on development *Pinus sylvestris* L.
Analysis seedlings *P. sylvestris* L. growth medium with copper nanoparticles model revealed significant changes of biometric parameters.

The experiment established that the presence of copper nanoparticles in the medium did not alter the germination of seeds, however, at the maximum experimental concentration, the length of the shoot was reduced and the formation of the root system was suppressed. Another factor testifying to the low tolerance of *P. sylvestris* L. to the effects of copper is a definite suppression of the frequency of germination, shoot length and roots in an experiment with the presence of copper ions in the medium. In a variant of the experiment with the presence of copper microparticles in the culture medium, an insignificant suppression of the growth of the root system was revealed. (table 2).
Table 2. The value of the upper limit of tolerance, mmol / l

| Investigated forms   | The parameters studied |   |
|----------------------|------------------------|---|
|                      | seed germination       | shoot growth | root growth |
| Cu ions              | 3.9                    | 3.3           | 0.2         |
| Cu nanoparticles     | >98                    | >98           | 1.6         |
| Cu microparticles    | >98                    | >98           | 6.8         |

The analysis of morphometric parameters of seedlings cultured in a medium with the presence of Cu nanoparticles revealed a significant change in the studied biometric parameters. Cu nanoparticles did not significantly change the germination rate of seeds, but at maximum concentration (100 mmol / l) they suppressed shoot growth, and to a greater extent inhibited root growth (1.6 mmol / l). In a variant of the study of the ionic form of copper, a lower tolerance was revealed, which affected seed germination (3.9 mmol / l), as well as a significant decrease in the length of the shoots (3.3 mmol / l) and the root system (0.2 mmol / l). The microparticles of copper did not have a significant inhibitory effect on the frequency of germination of seeds and the length of shoots, however, the formation of the root system (6.8 mmol / l) was slightly suppressed. The results of the study are confirmed by data on a similar issue of domestic scientists, who also note the phytotoxicity of Cu nanoparticles [2,11,18]. Global research confirms that the main effect of metal nanoparticles is on the root system of plants [5,9].

Table 3. Biometric indicators of seedlings of Pinus sylvestris L. when cultivated in the medium containing Cu nanoparticles

| Concentration (mmol / l) | Average length of main root (mm) | The average number of adventitious roots (pieces) | Average length of adventitious roots |
|--------------------------|----------------------------------|--------------------------------------------------|-------------------------------------|
| Control                  | 97.7±4.92*                       | 2.09±0.05*                                       | 75.4±3.8*                          |
| 100                      | 3.1±0.52*                        | 3.5±0.28*                                        | 2.12±0.17*                         |
| 25                       | 4.1±1.5                          | 3.7±0.3                                          | 2.37±0.23                          |
| 6.25                     | 16.0±1.94*                       | 3.9±0.22*                                        | 6.31±052*                          |
| 1.56                     | 44.9±3.82*                       | 2.66±0.18                                        | 23.61±1.62*                        |
| 0.39                     | 94.6±8.08*                       | 2.18±0.15*                                       | 67.21±4.59                         |

* p < 0.05

When P. sylvestris L. was cultivated in a medium containing Cu nanoparticles at concentrations of 0.025–0.1 M, the development of the root system was completely blocked. Cultivation in an environment with a content of Cu nanoparticles at a concentration of 6.25 mmol decreased the root length to 27.9 ± 3.8 mm, and also reduced the weight to 19.6 ± 1.9 mg.

In the control samples, the length of the root system was 219.8 ± 20.2 mm, and the mass was 56.4 ± 2.7 mg (tab. 3).

The study allowed to identify the phytotoxic effect of Cu in the series: germination - shoot growth - root growth, as well as assessing the parameter “growth and development of the root system” to reveal an increase in Cu activity in the series: microparticles - nanoparticles - ions.

Cytological analysis of prototypes of the P. sylvestris L. root system (apical part, root cap, primary meristem), cultivated at concentrations close to the upper tolerance limit, made it possible to identify certain features of the first samples. For example, cultivation in an environment with a high content of Cu nanoparticles affected the organization of cell walls. Microcopying revealed a large number of plasmodesmes, representing electron-dense bands that cross cell walls at a distance of 0.5-2.2 μm from each other. We assume that this fact may be a consequence of the passage of nanoparticles through them. Confirmation of the hypothesis is the size of plasmodesma (70 nm) and the size of nanoparticles (84 ± 5 nm). Not unimportant feature is the identified factor - the thickening of the cell walls of the root 2 or
more times (688 ± 119 nm). The revealed feature may indicate an adaptive response of P. sylvestris L. tissues to the entry and distribution of Cu nanoparticles.

4. Conclusion

The studies revealed a low tolerance of P. sylvestris L. to the action of Cu nano- and microparticles. The morphobiological studies of P. sylvestris L. grown in a cultured medium with the presence of Cu nanoparticles revealed significant morphobiological changes in the root system. The obtained data are consistent with the data of domestic and foreign authors on the effect of copper nanoparticles on grass species and algae [1-5]. At the same time, it was reliably established that the presence of Cu nanoparticles in the cultured medium, whose dimensions did not exceed 120 nm, did not affect the germination of seeds, however, at maximum concentrations, there was a pronounced effect of suppressing the formation of the root system and the stem, as well as a decrease in the root mass and an increase in its diameter in comparison with the control. Similar studies in herbaceous plants have shown that the phytotoxic effect of nanoparticles still manifests itself in inhibiting seed germination, reducing the length of shoots and roots, reducing the rate of photosynthesis and respiration, as well as morphological and enzymatic changes [6-11]. This fact indicates some stability of P. sylvestris L. to the content of copper nanoparticles in the environment. The lower tolerance of P. sylvestris L. was found to the ionic form of copper, as well as in grassy plants suppression of the frequency of seed germination was noted, along with a decrease in the length of the root system.

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