The impact of copper oxide and silver nanoparticles on woody plants obtained by *in vitro* method

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Abstract. The article substantiates the necessity of studying behaviour of copper oxide and silver nanoparticles on forest cultures (downy birch 29-58 (*Betula pubescens* Ehrh.) and poplar ‘Pyramidal-osokoreviy Kamyshinsky’ (*Populus pyramidalis* Roz. *x* *Populus nigra* L.) in *in vitro* as well as in ‘soil-plant-microbiota’ to ensure stability of forest cultures in forest regeneration. The impact of nanoparticles on shoot regeneration and propagation processes was evaluated by introducing nanoparticles into Woody Plant medium. Differences in the influence of nanoparticles on the life processes of plants depending on their concentration and the stage of clonal micropropagation have been established. The results are demonstrated by a 15-25% reduction in the frequency of infection of poplar and birch explants as well as by an increase in their regenerating potential at the stage of introduction in tissue culture. When the nanoparticle solution is used in the soil substrate, a decrease in the number of diseased plants and an increase in their survival rate of 30% can be observed. The inhibitory effect of silver nanoparticles on some ecological and trophic groups of microorganisms has been established. These results can be used in the application of CuO and Ag nanoparticles in the biotechnology of clonal micropropagation of forest crops.

1. Introduction

The enormous potential and unique properties of nanoparticles have provoked an increasing flow of research in recent years on their use in modern agriculture and forestry [1,2]. There are many conflicting opinions on the use of nanoparticles that have an effect on plant cells [3-5]. However, all researchers are inclined to believe that the effect of nanoparticles is present. The rapidly increasing variety of uses of nanoparticles raises the need to assess their impact on the environment, biota (microorganisms, plants, animals) and human health.

The use of metal nanoparticles in various stages of planting material cultivation by tissue culture has been shown for *Mentha longifolia* [6]. Modification of Murashige and Skoog culture medium (MS) with Cu (0.5 mg·l⁻¹) and Co (0.8 mg·l⁻¹) nanoparticles promoted intensification of multiplication process, and also improved the main morphometric parameters: the increase of plant height by 45-48.4%, number of internodes by 29.4-33.9%, number of shoots by 55.6-66.2%. Nanoparticles CuO (10 mg·l⁻¹) increased the level of organogenesis in rice culture by 94% [7] and stimulated seed...
germination in lettuce (*Lactuca sativa*) [8]. Higher rates of organogenesis and physiologically active glycoside synthesis in *Stevia rebaudiana* in vitro cultures were observed using MS culture medium containing CuO nanoparticles (10 mg l⁻¹) synthesized by coprecipitation [9]. In [10], the addition of Ag nanoparticles to *Tecomella undulata* culture medium at concentrations from 5 to 80 mg l⁻¹, alone or in combination with 6-benzylaminopurine and indolyl-3-acetic acid, was shown to increase the average number of new shoots, i.e., to enhance multiplication. The suggestion arises that the nanoparticle stimulation effect is related to their inhibitory effect on the major hormone ethylene, responsible for cell senescence. Improvement of the main growth parameters (seed germination, germination energy, seedling height) under the influence of Ag nanoparticles was also observed in *Boswellia ovalifoliolata* [11]. For the reproduction of callus cultures of *Caralluma tuberculata* to increase the yield of biomass and the production of phenolic compounds, flavonoids, 60 mg l⁻¹ Ag nanoparticles were added to MS based medium [12].

Having significant activity and the ability to influence many physiological processes of a living cell, nanoparticles entering the lithosphere can change the microbiological and enzymatic activity of soils due to interaction with living organisms living in this environment. There is a close relationship between microorganisms and plants. Any changes introduced into the microbiota of the plant rhizosphere will entail modifications aimed at the growth, development and productivity of plants [13]. The purpose of this work is to study the effect of copper nanoparticles and silver oxide on birch and poplar plants in culture in vitro and in the soil-plant-microbiota system. In addition, the possibility of using nanoparticles in clonal micropropagation technology of woody plants (poplar, birch) is planned to be established. It is predicted that the introduction of nanoparticles into the nutrient medium will enhance the growth and morphogenesis of microclones, as well as reduce the risk of microbial contamination of both explants and microstructures.

2. Methods and materials

The research was conducted at the Research Institute of Innovative Technologies and the Forestry Complex of the Voronezh State University Forestry and Technologies named after G.F. Morozov in the Polymerase Chain Reaction Laboratory (Voronezh, 8 Timiryazev Street, N51°43.129′, E39°13.264′) in spring and summer of 2020.

Copper oxide nanoparticles were prepared by the sol-gel method [14]. For this, 1 ml of glacial acetic acid was added to a 0.2 M aqueous solution of CuCl₂·2H₂O (Sigma-Aldrich, USA) and heated to 100°C. The pH was corrected to 7.0 with 8M NaOH (Sigma-Aldrich, USA) solution and the solution became black. The resulting precipitate was centrifuged (EBA 200, Hettich, Germany) and washed 3-4 times with deionized water. Then, we dried the resulting sludge for 24 hours. The colloidal solution was prepared by ultrasonic dispersion (S30H, Elma, Germany) of 20 mg powder in 100 ml distilled water (pH=7±0.2) for 5 minutes. Thus, a suspension with a nanoparticle concentration of 200 mg l⁻¹ was obtained.

Silver nanoparticles were obtained by chemical reduction [15]: in 50 ml of an aqueous solution containing 0.062 g (3.8·10⁻⁴ mol) AgNO₃ (≥98%, Sigma-Aldrich, USA), 70% dodecanolsulfoethoxylate (Sigma-Aldrich, USA) was added by drip method, stirring intensively, to 50 ml of an aqueous solution containing 0.2 g (5.2·10⁻⁴ mol) sodium salt (Sigma-Aldrich, USA). After 15 minutes of stirring the solutions, 100 ml of solution containing 0.028 g (7.4·10⁻⁴ mol) NaBH₄ (Sigma-Aldrich, USA) was added dropwise to the reaction system with vigorous stirring (C-MAG HS 7 digital IKAMAG, IKA-WERKE, Germany). The concentration of silver in the resulting dispersion was 200 mg l⁻¹.

Electron microscopic study was carried out on a Merlin high-resolution scanning electron microscope (SEM) (Carl Zeiss, Germany). Particle size analysis in colloidal systems was performed using a Zetasizer Nano system (Malvern Instruments, UK).

Apical and axillary meristems of young poplar ‘Pyramidal-osokoreviy Kamyskhinsky’ (*Populus pyramidalis Roz. x Populus nigra* L.) and downy birch (29-58) (*Betula pubescens* Ehrh.) shoots were used as explants for in vitro tissue culture. The shoots were disinfected according to the generally
accepted method [16,17]. Sterile shoots were cut in aseptic conditions into 1.5-2.0 cm segments with one axillary bud – explants, which were then planted on agar culture medium WPM (Woody Plant Medium) [18] with addition of CuO and Ag nanoparticles solutions at concentrations of 5 and 10 mg·l⁻¹. The medium without nanoparticles was used as a control. The flasks with explants were placed in a growing room with climatic conditions: 16-h photoperiod with an illumination of 2-3 klx, and a temperature of 24-26 °C. The number of contamination-free explants and regenerating explants that formed the main shoot were recorded for 21 days.

The impact of nanoparticle solutions on regeneration processes as well as the reproduction rate of poplar and birch microshoot was evaluated on WPM medium with the addition of nanoparticles as well as growth regulators – 300 μg·l⁻¹ benzylaminopurine (BAP) (Acros Organics, USA) and 200 μg·l⁻¹ gibberellic acid (GA) (Acros Organics, USA). The growth-regulating activity of the nanoparticles after 21 days was evaluated according to the following parameters: viability, appearance, shoot and leaf development, and multiplication index. The condition of the microshoots was evaluated by their appearance on a 5-point scale. The multiplication index was calculated as the average number of shoots obtained from one conglomerate.

The process of shoot rooting under the influence of nanoparticle solutions was carried out on ½ WPM hormone culture medium supplemented with 300 μg·l⁻¹ of indolyl-3-acetic acid (IAA) (Acros Organics, USA). By the resulting of 21 days of the experiment was established that, the number of shoots that had formed roots as well as the number of roots per shoot were counted.

At the stage of transferring microplants to non-sterile greenhouse conditions for 21 days the effect of nanoparticles on such basic indicators as survival rate of poplar and birch microplants, presence of infection as well as morphometric indicators (stem height, number of leaves, general plant condition) were revealed. The substrate, consisting of peat and perlite at a ratio of 3:1, was treated with a solution of nanoparticles to a final concentration of 5 mg·l⁻¹. The soil mixture was poured into 150 cm³ picking containers into which the plants were planted.

Studies with plants were carried out in 5-fold replications. For each variant 100 (20×5) plants were taken. The data were statistically processed by analysis of variance using Microsoft Excel 2010 at 5% significance level.

The antagonistic activity of the cultures against phytopathogens Fusarium oxysporum, Fusarium avenaceum, Alternaria alternata, and representatives of beneficial soil microflora (Azotobacter sp., Azospirillum sp., Pseudomonas fluorescence, Bacillus sp., Bacillus mycoides, Streptomyces sp.) was determined in Petri dishes using the holes method [19]. For this purpose, nutrient medium suitable for the development of the corresponding microorganism was poured into Petri dishes: for Fusarium oxysporum, Fusarium avenaceum, Alternaria alternata, Streptomyces sp. – Czapek’s medium medium, Azotobacter sp., Azospirillum sp. – Ashby’s medium, Pseudomonas fluorescence, Bacillus sp., Bacillus mycoides - MPA. The dishes were inoculated with a culture of microorganisms. Holes were cut in the agar with a sterile microbial drill and 100 μl each of CuO, Ag nanoparticle solutions (5 mg·l⁻¹) were added. The plates with cultures were incubated in an incubator at 28 °C for 4 days. The degree of antagonistic activity was evaluated by the zones of test culture growth inhibition around the holes with the studied nanoparticles.

3. Results and discussion
It was shown by scanning electron microscopy that copper oxide nanoparticles have a columnar structure, with an average size of individual columns of 50×200 nm (figure 1a). Silver nanoparticles have a spherical shape with a size of 30-60 nm (figure 1b).
Figure 1. Scanning electron microscope images of CuO (a) and Ag (b).

The analysis of the disperse composition by the method of dynamic light scattering (DLS) of a colloidal solution of copper oxide nanoparticles showed that the average particle size in the suspension is 50-70 nm (figure 2a). The average size of silver nanoparticles in the dispersion was in the range of 10-20 nm (figure 2b). The decrease in particle size in comparison with the SEM data is probably associated with the ultrasonic treatment of the solutions.

Figure 2. Particle size in colloidal systems CuO (a) and Ag (b).

Obtaining planting material of woody plants by clonal micropropagation is a well-defined process consisting of several stages: creation of an aseptic culture (introduction of tissues into the culture \textit{in vitro}); reproduction (proliferation); rhizogenesis; transfer of plants to non-sterile conditions (adaptation or acclimatization). In the first series of experiments, the effect of nanoparticle solutions on woody plants was carried out on poplar and birch plants at different stages of the \textit{in vitro} micropropagation process.

At the stage of introducing woody plants into tissue culture (\textit{in vitro}), the use of CuO and Ag nanoparticles in the cultivation medium was shown to increase the number of contamination-free explants in both birch and poplar (table 1). The optimum concentration of nanoparticles at which the number of contamination-free explants was maximum was 5 mg·liter$^{-1}$. Using this concentration of nanoparticles in the cultivation medium made it possible to achieve up to 90% of sterile explants in poplar (control 65%) and 95% in birch (control 30%).
Table 1. The basic indicators of poplar (POK) and downy birch (29-58) explants depending on the addition of nanoparticles.

| Plant in vitro | Nanoparticles in the WPM cultivation medium | Indicators | Number of contamination-free explants (%) | Number of regenerating explants (%) |
|---------------|--------------------------------------------|------------|------------------------------------------|-----------------------------------|
| Poplar (POK)  | CuO – 5 mg·l⁻¹                           | 90.0±5.2   | 70.0±5.0                                 |
|               | CuO – 10 mg·l⁻¹                          | 90.0±5.7   | 15.0±3.5                                 |
|               | Ag – 5 mg·l⁻¹                            | 80.0±6.5   | 45.0±3.5                                 |
|               | Ag – 10 mg·l⁻¹                           | 90.0±3.5   | 30.0±7.1                                 |
|               | control                                  | 65.0±5.2   | 40.0±7.1                                 |
| Downy birch   | CuO – 5 mg·l⁻¹                           | 85.0±3.2   | 85.0±3.2                                 |
|               | CuO – 10 mg·l⁻¹                          | 80.0±6.5   | 80.0±3.5                                 |
|               | Ag – 5 mg·l⁻¹                            | 95.0±3.2   | 95.0±3.2                                 |
|               | Ag – 10 mg·l⁻¹                           | 60.0±5.0   | 65.0±6.3                                 |
|               | control                                  | 60.0±7.1   | 65.0±6.3                                 |

One of the main indicators to be considered when choosing a sterilising agent is the number of regenerating explants, i.e., explants that have formed a well-developed main shoot. CuO and Ag nanoparticles used as disinfecting agents in WPM medium had a positive effect on the regenerative ability of explants, which was studied by the development of the main shoot formed from the bud (table 1). The degree of influence depended on the concentration of nanoparticles used. Thus, when CuO nanoparticles were used in WPM medium at a concentration of 5 mg·l⁻¹, the number of poplar explants which developed the main shoot (regenerating explants) was 70%, while in the control - 40%. A twofold increase in concentration contributed to a 25% decrease in this index from the control, indicating the inhibitory effect of the solutions of the studied nanoparticles on the embedded plant tissues. In addition, this concentration caused necrosis of explants. Similar data were obtained with Ag nanoparticles; increasing the concentration of nanoparticles led to a 10% decrease in the number of regenerating explants. Based on visual observation, it was observed that explants grown in medium supplemented with nanoparticles at a concentration of 10 mg·l⁻¹ regenerated significantly slower. When the number of regenerating birch explants was analysed, it was found that the use of CuO and Ag nanoparticles at a concentration of 5 mg·l⁻¹ increased their number by 20 and 30%, respectively. Increasing the concentration of nanoparticles to 10 mg·l⁻¹ increased this index only by 15%. Thus, the most optimal concentration of nanoparticles for main shoot formation in poplar and birch explants was found to be 5 mg·l⁻¹ (figure 3).

Figure 3. Impact of nanoparticles (5 mg·l⁻¹) in WPM culture medium on the development of the main shoot on poplar (POK) (a) and downy birch (29-58) explants (b) after 30 days from introduction into tissue culture.
The propagation stage is the longest, in which microshoots propagate cyclically: the planted explant turns into a conglomerate of shoots after a few weeks, which are divided into individual explants and planted again on a new cultivation medium. One of the main objectives at this stage, which research scientists have to achieve a maximum number of shoots from a single conglomerate. In our study it was found that CuO and Ag nanoparticles (5 mg·l⁻¹) have no propagative effect on poplar and birch microshoots. Table 2 shows that the presence of CuO and Ag nanoparticles in the cultivation medium had a negative effect on the overall condition of poplar shoots (3.2 and 4.1 points, respectively), the control – 4.4 points. At the same time, poplar shoot height decreased by 60 and 53%, and the number of leaves by 7 and 49%, respectively. Visual observations showed that the shoots looked weak, with elongated internodes. CuO and Ag nanoparticles at a concentration of 5 mg·l⁻¹ had no effective propagation effect on poplar plants: the propagation index was 1.6 and 1.1, respectively (1.6 in the control). The use of Ag nanoparticles in the cultivation medium had the effect of lengthening the birch shoots by 10%, but the number of leaves and the multiplication index decreased. The plants lacked the intense green colour characteristic of healthy plants, looked weak, with thin shoots, elongated internodes, and their condition was rated as 3.7 on a 5-point scale.

Table 2. The basic poplar (POK) and downy birch (29-58) shoots as a function of nanoparticle addition after 21 days of cultivation.

| Plant in vitro | Nanoparticles | Shoot height, mm | Number of leaves per plant | Multiplication index | General condition of shoots on a 5-point scale |
|---------------|---------------|------------------|----------------------------|----------------------|-----------------------------------------------|
| POK           | CuO           | 11.6±1.4         | 6.6±0.7                    | 1.6±0.2              | 3.2±0.3                                       |
|               | Ag            | 13.7±0.8         | 3.6±0.2                    | 1.1±0.1              | 4.1±0.1                                       |
|               | Control       | 29.3±2.6         | 7.1±0.5                    | 1.6±0.2              | 4.4±0.2                                       |
| Downy Birch   | CuO           | 21.3±2.0         | 6.4±0.7                    | 1.7±0.1              | 3.0±0.2                                       |
|               | Ag            | 27.1±2.8         | 3.6±0.4                    | 1.8±0.2              | 3.7±0.2                                       |
|               | Control       | 25.9±2.2         | 6.9±0.9                    | 2.5±0.3              | 4.5±0.1                                       |

An important step in clonal micropropagation technology is the stage of rhizogenesis of microshoots. The nanoparticles tested at a concentration of 5 mg·l⁻¹ showed no root-forming effect on mericlones (table 3). On nutrient medium ½ WPM, optimal for rooting of woody plant microshoots, containing IAA (300 μg·l⁻¹) as a root-forming stimulant in the control version, after 21 days of observation the number of poplar shoots that formed a root system was 65%. On medium with CuO and Ag nanoparticles this figure was lower and amounted to 40 and 50%, respectively. When rhizogenesis in birch was studied under the influence of nanoparticles, the number of rooted shoots was 35 (with the addition of CuO) and 60% (with the addition of Ag), while in the control version it was 75%. However, it was noted that the addition of CuO nanoparticles to the nutrient medium for rhizogenesis process increased the number of roots formed on each plant. Thus, on poplar shoots the number of roots averaged 2.2 per shoot (control – 1.3), and on birch – 3.6 (control – 3.1).

Table 3. Impact of nanoparticles on rhizogenesis of poplar (POK) and downy birch shoots (29-58).

| Plant in vitro | Indicators                              | Nanoparticles in the cultivation medium |
|---------------|-----------------------------------------|----------------------------------------|
|               |                                        | CuO         | Ag          | Control     |
| Poplar (POK)  | Percentage of rooted microclones        | 40.0±9.2    | 50.0±3.5    | 65.0±7.9    |
|               | Number of roots per plant               | 2.2±0.7     | 1.3±0.3     | 1.3±0.1     |
| Downy birch   | Percentage of rooted microclones        | 35.0±5.2    | 60.0±7.1    | 75.0±9.2    |
|               | Number of roots per plant               | 3.6±0.6     | 2.5±0.4     | 3.1±0.4     |

At the stage of adaptation of poplar and birch to non-sterile soil conditions, microplants propagated by tissue culture method adapt to greenhouse and field conditions - they create their own root system,
functional mechanism of transpiration, protective tissue layers and begin their effective process of photosynthesis. In order to reduce the proportion of dead healthy plants at the stage of adaptation to non-sterile environmental conditions, we tested nanoparticle solutions in the soil substrate. The effect of CuO and Ag nanoparticle solutions on the survival rate and the presence of infection in poplar and birch micro-plants 21 days after their transfer to room conditions are presented in table 4.

Table 4. Main indices of poplar (POK) and downy birch (29-58) plants depending on the addition of nanoparticles during the adaptation phase to non-sterile greenhouse conditions.

| Plant ex vitro | Nanoparticles | Stem height, mm | Number of leaves per plant | Survival rate, % | Number of diseased plants, % | General condition of plants on a 5-point scale |
|---------------|---------------|-----------------|---------------------------|-----------------|-------------------------------|-----------------------------------------------|
| POK           | CuO           | 45.2±7.4        | 6.5±0.5                   | 75.0±8.5        | 5.0±1.6                       | 4.5±0.2                                      |
|               | Ag            | 43.9±4.1        | 6.8±0.4                   | 100             | 0                             | 5.0                                           |
|               | control       | 30.0±6.3        | 4.2±0.3                   | 70.0±7.1        | 10.0±3.5                      | 4.3±0.2                                      |
|               | CuO           | 47.8±3.3        | 5.9±0.2                   | 95.0±2.7        | 5.0±1.6                       | 4.6±0.1                                      |
|               | Ag            | 50.7±5.4        | 6.4±0.4                   | 95.0±3.9        | 10.0±1.6                      | 4.6±0.2                                      |
|               | control       | 45.0±3.9        | 4.4±0.3                   | 70.0±5.7        | 15.0±2.2                      | 4.1±0.3                                      |

The data presented in Table 4 show that when CuO and Ag nanoparticles (5 mg·l⁻¹) were added to the soil substrate, 75 and 100% of the poplar plants acclimated in the greenhouse had higher initial growth and development parameters. It was found that the highest stem height (45.2 and 43.9 mm), number of leaves (6.5 and 6.8 pieces per plant) were achieved in poplar micro-plants transferred to substrate treated with CuO and Ag nanoparticles, respectively. In control plants these values were 30.0 mm and 4.2 leaves per plant. The condition of the plants was rated as 4.5 and 5.0 on a 5-point scale. Due to the high disinfecting effect of the investigated nanoparticles against pathogens, the number of plants with signs of disease was reduced. Thus, when CuO nanoparticles were added to the substrate, only 5% of plants with signs of disease were found (control 10%), while when Ag nanoparticles were added, not a single diseased plant was detected. The nanoparticles in the soil substrate also contributed to a 25% increase in the survival rate of birch plants. The best micro-plant growth was observed in the substrate containing nanoparticles. An improvement in the overall condition of the plants was observed, which was rated at 4.6 on a 5-point scale, with the experimental plants outperforming the control plants in growth and development. This fact of positive effect indicates an increase in the adaptive properties of birch micro-plants under the influence of nanoparticle solutions, which is consistent with the literature data. According to many researchers [20, 21], the use of nanoparticles reduces the effects of stress factors, which include the transfer of plants to greenhouses. At the same time, nanoparticles act on biological objects at the cellular level, increasing the efficiency of processes occurring in plants, and have a prolonged effect.

Thus, the result obtained shows that the use of nanoparticles at the stage of transferring poplar and birch plants to greenhouse growing conditions can increase the survival rate of tree plant microclones and reduce the risk of microbial infection by introducing CuO and Ag nanoparticle solutions into the technological process.

The second series of studies examined the ability of nanoparticles to influence the functioning of beneficial and phytopathogenic soil microflora in the soil. Soil microflora plays a significant role in the structure of terrestrial biocenoses [22]. It is known that microorganisms determine the ecological state of the soil as the environment of their functioning and reproduction. Analysis of the effect of nanoparticles on phytopathogens (table 5) showed significant antifungal effect of CuO (5 mg·l⁻¹) against *Fusarium oxysporum*, *Alternaria alternata* and Ag against *Fusarium avenaceum* and *Alternaria alternata*. This property of nanoparticles to suppress the source of infection is important because the pathogens of major diseases of woody plants for a long time in the form of microsclerotia can persist in the soil and penetrate into the root system, causing the disease. The presence of a
significant antifungal effect explains the decrease in the number of infected poplar and birch explants at the initial stage of the process of introducing plants into in vitro culture, as well as the decrease in the number of diseased plants when transferring micro-plants for cultivation in greenhouse conditions.

Table 5. Impact of nanoparticles on phytopathogenic soil microflora.

| Nanoparticles | Testing microorganism          |
|---------------|--------------------------------|
|               | *Fusarium avenaceum* | *Fusarium oxysporum* | *Alternaria alternata* |
| CuO           | 0 | + | + |
| Ag            | ++ | 0 | + |

*0* – no growth suppression, *+* – growth suppression of the testing microorganism

The experimental data were reflected in table 6 show that CuO nanoparticles (5 mg l⁻¹) do not inhibit the growth of the beneficial bacteria tested, indicating that they are biologically harmless.

Table 6. Impact of nanoparticles on beneficial soil microflora.

| Nanoparticles | Testing microorganism          |
|---------------|--------------------------------|
|               | *Azotobacter sp.* | *Pseudomonas fluorescens* | *Bacillus sp.* | *Azospirillum sp.* | *Bacillus mycoides* | *Streptomyces sp.* |
| CuO           | 0 | 0 | 0 | 0 | 0 | 0 |
| Ag            | + | + | + | ++ | + | ++ |

*0* – no growth suppression, *+* – growth suppression of the testing microorganism

It was found that Ag nanoparticles suppress the growth of nitrogen-fixing organisms (*Azotobacter sp.*, *Azospirillum sp.*), plant growth and development stimulators (*Pseudomonas fluorescens*, *Bacillus sp.*), as well as actinomycetes involved in complex transformations of organic matter in soil. This fact dictates the need for further in-depth research on the selection of optimal concentrations of these nanoparticles when applied to soil in order to reduce the infectious load of phytopathogens on poplar and birch microplants.

4. Conclusion

The results of our research have led us to conclude:

When introduced into in vitro culture of birch and poplar to reduce the level of infection load it is advisable to introduce solutions of CuO and Ag nanoparticles as sterilizing agents in the cultivation medium at a concentration of 5 mg l⁻¹, with the number of uninfected explants was 80-95% (60-65 % in the control). It was noted that the nanoparticles used in the experiment also contributed to the increased regenerative ability of explants.

Addition of nanoparticles to soil substrates at a stage of plant transfer into greenhouse conditions was shown to reduce the number of plants infected by phytopathogens by 5-10% and to increase the survival rate by 5-30%. Experimental birch and poplar plants had improved growth and development due to the reduction of infectious onset in the substrate under the influence of nanoparticles.

The application of CuO and Ag nanoparticles (5 mg l⁻¹) at the stages of propagation and rhizogenesis of poplar and birch microshoots had no positive effect. Due to the fact that the number of roots on the plant has increased in the case of the use of CuO nanoparticles at the stage of rhizogenesis, there is a need for more detailed studies of the mechanisms of action of nanoparticles on physiological processes in microclonal plants.

Significant antifungal effect of CuO and Ag nanoparticles against major pathogens of woody plants was established. However, in addition to the antifungal effect, Ag nanoparticles exhibit an inhibitory effect on most representatives of beneficial soil microflora.

Thus, to intensify the process of clonal micropropagation of woody plants, the possibility of using nanoparticles of copper and silver oxide at the stages of introduction to the culture in vitro and during
the transfer of micro-plants in greenhouse conditions was established. The use of nanoparticles has improved the in vitro propagation technology of woody plants (poplar, birch), which can more fully realize their biological potential and provide accelerated serial production of high-quality planting material on an industrial basis.

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References
[1] Zakharova O, Kolesnikov Е, Vishnyakova E, Strekalova N and Gusev A 2019 Antibacterial activity of ZnO nanoparticles: dependence on particle size, dispersion media and storage time. *IOP Conf. Ser.: Earth Environ. Sci.* 226 012062 doi: 10.1088/1755-1315/226/1/012062

[2] Bundschuh M, Filser J, Ludermald S, McKee M, Metreveli G, Shaumann G, Schultz R and Wagner S 2018 Nanoparticles in the environment: where do we come from, where do we go to? *Environ. Sci. Eur.* 30(6) 1 doi: 10.1186/s12302-018-0132-6

[3] Dikman L and Shchyogolev S 2017 Interactions of plants with noble metal nanoparticles (review). *Agricultural biology* 52(1) 13 doi: 10.15389/agrobiology.2017.1.13eng

[4] Ben-Moshe T, Frenk S, Dror I, Minz D and Berkowitz B 2013 Effects of metal oxide nanoparticles on soil properties. *Chemosphere* 90(2) 640 doi: 10.1016/j.chemosphere.2012.09.018

[5] Carriere M and Larue C 2012 Toxicology: plants and nanoparticle *Encyclopedia of Nanotechnology* (New York: Springer) chapter 182 pp 2763-2767

[6] Talankova-Sereda T E, Liapina K V, Shkopinskij E A, Ustinov A I, Kovalyova A V, Dulnev P G and Kucenko N I 2016 The influence of Cu and Co nanoparticles on growth characteristics and biochemical structure of *Mentha longifolia in vitro*. *Nanoscience and Nanoengineering* 4(2) 31 doi: 10.13189/nn.2016.040201

[7] Anwaar S, Maqbool Q, Jabeen N, Nazar M, Abbas F, Nawaz B, Hussain T and Hussain S 2016 The effect of green synthesized CuO nanoparticles on callogenesis and regeneration of *Oryza sativa L*. *Front. Plant. Sci.* 7(535) 1330 https://doi.org/10.3389/fpls.2016.01330

[8] Shah V and Belozerova I 2009 Influence of metal nanoparticles on the soil microbial community and germination of lettuce seeds. *Water Air. Soil Poll.* 197(1) 143 https://doi.org/10.1007/s11270-008-9797-6

[9] Javed R, Mohamed A, Yucesan B, Gurel E, Kausar R and Zia M 2017 CuO nanoparticles significantly influence *in vitro* culture, steviol glycosides, and antioxidant activities of *Stevia rebaudiana* Bertoni. *Plant. Cell. Tiss. Org.* 131(1) 611 doi:10.1007/s11240-017-1312-6

[10] Aghdai M, Salehi H and Sarmast M 2012 Effects of silver nanoparticles on *Tecomella undulata* (Roxb.) Seem. Micropropagation. *Advances in Horticultural Science* 26(1) 21 doi:10.13128/ahs-12748

[11] Savithramma N, Ankanna S and Bhumi G 2012 Effect of nanoparticles on seed germination and seedling growth of *Boswellia ovalifoliolata* an endemic and endangered medicinal tree taxon. *Nano Vision* 2(1-3) 61

[12] Ali A, Mohammad S, Khan M A, Raja N I, Arif M, Kamil A and Mashvani Z R 2019 Silver nanoparticles elicited *in vitro* callus cultures for accumulation of biomass and secondary metabolites in *Caralluma tuberculata*. *Artif. Cell. Blood. Sub.* 47(1) 715 https://doi.org/10.1080/21691401.2019.1577884

[13] Navratilova J, Praetorius A, Gondikas A, Fabenke W, von der Kammer F and Hofmann T 2015 Detection of engineered copper nanoparticles in soil using single particle ICP-MS. *Int. J. Env. Res. Pub. He.* 12(12) 15756 https://doi.org/10.3390/ijerph121215020
[14] Kshirsagar J, Shrivastava R and Adwani P 2017 Preparation and characterization of copper oxide nanoparticles and determination of enhancement in critical heat flux. *Therm. Sci.* **21**(1) 233 https://doi.org/10.2298/tsci140619026k

[15] Iravani S, Korbekandi H, Mirmohammadi S V and Zolfaghari B 2014 Synthesis of silver nanoparticles: chemical, physical and biological methods. *Research in Pharmaceutical Sciences* **9**(6) 385 https://doi.org/10.1007/978-3-319-14502-0_11

[16] Sereda M M, Tutschke E V, Chokheli V A, Vereschagina A V, Rashkovskaya K Y, Lysenko V S and Varduny T V 2017 A method for microclonal propagation of *Staurogyne repens* in tissue culture. *Journal of Plant Sciences* **12** 17 doi: 10.3923/jps.2017.17.21

[17] Evlakov P, Fedorova O, Grodetskaya T, Zakharova O, Gusev A, Krutyakov Yu and Baranov O 2020 Influence of Copper Oxide and Silver Nanoparticles on Microclonal Sprouts of Downy Birch (*Betula pubescens* Ehrh.). *Nanotechnologies in Russia* **15**(7-8) 476 doi:10.1134/S1995078020040035

[18] Lloyd G and McCown D 1980 Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia* by use shoot tip culture. *IPPS* **30** 421

[19] Jin T, Sun D, Su J Y, Zhang H and Sue H J 2009 Antimicrobial efficacy of zinc oxide quantum dots against *Listeria monocytogenes, Salmonella enteritidis, and Escherichia coli* O157:H7. *J. Food. Sci.* **74**(1) 46 doi: 10.1111/j.1750-3841.2008.01013.x

[20] Elmer W, Ma Ch and White J 2018 Nanoparticles for plant disease management. *Current Opinion in Environmental Science and Health* **6** 66 https://doi.org/10.1016/j.coesh.2018.08.002

[21] Liu R and Lal R 2015 Potentials of engineered nanoparticles as fertilizers for increasing agronomic productions. *Sci. Total. Environ.* **514** 131 https://doi.org/10.1016/j.scitotenv.2015.01.104

[22] Manchikanti P 2010 Nanomaterials and effects on biological systems: development of effective regulatory norms. *Nanoethics.* **4**(1) 77 https://doi.org/10.1007/s11569-010-0084-9