Concentration-dependent stimulating and toxic effects of ZrS$_3$ and TiS$_3$ nanoribbons on forest woody plants in tissue culture \textit{in vitro}

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Abstract. Nanotechnology has a great potential for application in applied biotechnology. Here we demonstrate the effectiveness of synthesized by direct reaction ZrS$_3$ and TiS$_3$ nanoribbons as sterilizing agents, growth stimulators and activators of rhizogenesis of micro-sprouts of tree crops during clonal micropropagation. At the initiation stage at 6 and 15 μg/L ZrS$_3$ and 3, 6 and 15 μg/L TiS$_3$, complete sterility of shoots of brittle willow, red oak and Scots pine was noted. The maximum survival rate and seedling height at this stage was in the groups of 1.5 μg/L ZrS$_3$ and 3 μg/L TiS$_3$. An increase in the concentration of nanomaterials to 15 μg/L significantly reduced the viability of plants. At the proliferation stage the concentration of nanomaterials 1.5 and 3 μg/L increased the survival rate of regenerants, and at 3 μg/L with the phytohormones (benzylaminopurine, indoleacetic acid, gibberellic acid) the number of additional shoots increased. At the rooting stage ZrS$_3$ and TiS$_3$ at doses of 1.5 and 3 μg/L with auxin activated rhizogenesis, significantly increasing the number of seedlings with roots in comparison with the variants where only auxin were used. This effects can be associated both with the direct action of nanoribbons and with the release of hydrogen sulfide as a result of aqueous hydrolysis of nanoribbons, since H$_2$S plays an important role in the regulation of plant physiological processes.

1. Introduction
Micropropagation is an effective method of \textit{in vitro} vegetative propagation of plants. The advantage of the method is the ability to quickly obtain genetically stable clone plants identical to the parent plant. The effectiveness of tissue culture \textit{in vitro} is associated with factors such as the initial genotype, physiological state of mother plants, disinfection technique, composition of the nutrient medium, and plant growth regulators. At the initiation stage of tissue culture, great difficulties are associated, most often, with a high infection of explants. Considering that current sterilization methods are not safe for
microclones, there is a need to search for new sterilizing agents, as well as growth stimulants for in vitro use [1].

Recent studies have shown that the use of nanoparticles in micropropagation of plants led to the elimination of microbial contaminants from explants and had a positive effect on the induction of the formation of callus and plant organs, cell proliferation, genetic homogeneity, and the physiological state of clones [2, 3].

According to the Hesami et al. [4], when chrysanthemum explants were immersed for 10 min in 10 mg/L of silver nanoparticles, the frequency of contamination was 15.56%, and the viability of explants was 84.44%. Arab et al. used 100 and 150 ppm Ag nanoparticles in immersion and in the Murashige and Skoog (MS) culture medium and observed significantly reducing of phytopathogen contamination of the almond × peach hybrids [5]. 10 mg/L of silver nanoparticles in MS medium increased the average number of new shoots per explant, the percentage of explants with shoots, and the plant survival rate. [6]. The authors associate these effects with the interaction of nanosilver with the phytohormonal regulation system, in particular, the blocking of ethylene receptors.

During the cultivation of B. nigra micro-sprouts on MS medium modified with 1–20 mg/L of ZnO nanoparticles, the formation of roots with developed root hairs was observed. Seedling growth was also observed at 10 mg/L ZnO [7]. Nanoparticles of copper (0.5 mg/L) and cobalt (0.8 mg/L) in the composition of the MS medium increased the height and growth index of M. longifolia clones by 45-48.4%, the number of seedlings by 55.6% -66.2%, reproduction rate by 30-40% [8]. The authors of the work [9] observed the rhizostimulating and antimicrobial effect of copper oxide nanoparticles on tissue culture of hybrid poplar × aspen. Evlakov et al. [10] used nanoparticles of copper oxide (5–40 nm) and silver (10–30 nm) for clonal micropropagation of downy birch. Nanoparticles into the woody plant medium (WPM) at the initiation stage reduced the infection rate of explants by 15–25%, as well as increased their growth rate. When birch sprouts were transferred to non-sterile conditions, nanoparticles colloids reduced the number of infected plants and increased their survival rate by 25%.

Our previous studies have shown a positive effect of zirconium trisulfide on tree shoots at the stage of adaptation to non-sterile soil conditions [11], as well as titanium trisulfide during microcloning of birch and hybrid white poplar × aspen [12]. In this regard, in this study, we analyze the effect of zirconium and titanium trisulfides on microclones of other common woody cultures at different stages of clonal micropropagation in order to search for new effective nanostimulants for micropropagation of woody plants.

2. Materials and methods
To assess the effect of nanomaterials on tree crops during clonal micropropagation, nanoribbons of zirconium trisulfide (ZrS₃) and titanium trisulfide (TiS₃) were used. To synthesize ZrS₃ and TiS₃ compounds, weighed portions of pure zirconium / titanium powder and elemental sulfur powder (≥99%, Sigma-Aldrich) were taken in a stoichiometric ratio in accordance with reactions (1) and (2) [13, 14].

\[
\begin{align*}
Zr + 3S &= ZrS_3 \\
Ti + 3S &= TiS_3
\end{align*}
\]

The obtained images were analyzed by scanning electron microscopy (SEM) (Vega3 Tescan, Czech Republic) and Raman spectroscopy (Thermo DXR, Thermo Scientific, USA). Experiments were conducted using the equipment of the Derzhavin State University Center for Collective Usage (Tambov, Russia).

The analysis of the impact of ZrS₃ and TiS₃ was carried out on micro-sprouts of brittle willow (Salix fragilis L., 1753), red oak (Quercus rubra L., 1753) and scots pine (Pinus sylvestris L., 1753) at various stages of cloning micro propagation: initiation, proliferation and rooting.

To carry out the study, ZrS₃ and TiS₃ nanoribbons were introduced into the composition of the cultivation medium. At the initiation stage, cuttings of plants with apical and axillary buds were used. The selected plant material was washed with running water using Tween-20, and then thoroughly rinsed with distilled water. Shoots were sterilized for 40 minutes in a solution consisting of 200 ml of distilled
water and 200 μl of 5% sodium hypochlorite solution, followed by washing in distilled water. The main sterilization of shoots was carried out in an aqueous solution of mercury chloride (HgCl₂) for 10–20 min in a laminar flow cabinet. Washing was also carried out with sterile water. Sterile shoots were cut under aseptic conditions into 1.5-2 cm segments with one axillary bud, after which the explants were planted on agar woody plant medium (WPM) [15], containing 0.75; 1.5; 3; 6 and 15 μg/L ZrS₃ / TiS₃. Tubes with explants were placed on the racks of the light installation of the culture room at a temperature of +24 °C, a day / night photoperiod of 16/8 h, illumination of 5000 lx, and a relative humidity of 70%.

After four weeks of cultivation, an analysis of the sterility and survival of the plants, as well as the morphometric parameters of the plants, including the plant height, the number of leaves, and the number of additional shoots, was carried out.

For the multiplication stage, normally developed microclones were selected, cuttings and transplanted into nutrient media of the following composition: MS0 - control, MS0 + nanoribbons, MS0 + hormonal components (PhH) (benzylaminopurine (BAP) - 0.2 mg/L, indoleacetic acid (IAA) - 0.1 mg/L and gibberelic acid (HA) - 0.3 mg/L), as well as MS0 + nanoribbons + PhH. After a month of cultivation, the morphometric parameters of the seedlings were assessed.

To initiate the rooting process, the regenerants of woody crops that reached a height of 2 - 3 cm were isolated and transferred to a medium for rooting - ½ WPM in culture vessels. To stimulate rhizogenesis, nanomaterials were also added to the nutrient medium at a concentration of 3 μg per liter of the nutrient medium and auxin - indolebutyric acid (IBA) at concentrations of 0.2 and 0.4 mg/L. At the end of the experiment, the number of microclones with roots was analyzed.

All biological and biochemical parameters were measured in three biological and analytical repetitions. Each experimental and control group at each stage of the study contained 30 plants. Descriptive statistics methods included an estimate of the arithmetic mean (M) and standard deviation (S). For statistical data processing, Microsoft Excel 2010 (Descriptive Statistics software package) with one-way analysis of variance (ANOVA) at a 5% predetermined level of significance was used.

3. Results and discussion

3.1. Study of ZrS₃ and TiS₃ nanoribbons samples

It has been founded by scanning electron microscopy that the resulting nanomaterials have a characteristic layered ribbon shape [16, 17] (figure 1).

![Figure 1. SEM images of: a) zirconium trisulfide; b) titanium trisulfide.](image)

The size of individual ZrS₃ ribbons (figure 2a) was as follows: thickness – less than 100 nm, length – more than 10 μm, and the width is about of 0.5 – 10 μm; TiS₃ (figure 2b) – thickness 100 nm, length – from 1 to 10 μm, and width is about of 0.4 – 1 μm.
The Raman spectra (figure 2 a, 2b) show lines specific for zirconium trisulfide [18, 19] and titanium trisulfide [20, 21], which confirms the presence of these phases in the obtained samples.

![Raman spectra of ZrS₃ (a) and TiS₃ (b).](image)

**Figure 2.** Raman spectra of ZrS₃ (a) and TiS₃ (b).

### 3.2. Micropropagation

#### 3.2.1. Initiation stage.

Evaluation of the state of brittle willow seedlings at the initiation stage under the influence of ZrS₃ showed the sterilizing effect of the nanomaterial at concentrations above 0.75 µg/L (figure 3a). At 1.5 µg/L, the number of sterile seedlings was 90%, while 78% in the control variant. An increase in the ZrS₃ concentration to 3 µg/L and higher promoted 100% sterility of seedlings. With a favorable effect of ZrS₃ on the sterility of plants, a multidirectional effect of nanoribbons on the survival of microclones was noted (figure 3a). Thus, at 1.5 µg/L, the number of surviving seedlings increased by 12%, and at 3 µg/L, by 22%, relative to the control. At the same time, an increase in the content of nanomaterial in the cultivation medium to 6 and 15 µg/L had a negative effect on willow seedlings, the survival rate decreased by 22 and 28%, respectively. A dose of 0.75 µg/L had no significant effect on the development of microclones.

![Sterility and survival of willow micro shoots under the effect of ZrS₃ (a) and TiS₃ (b) nanomaterials.](image)

**Figure 3.** Sterility and survival of willow micro shoots under the effect of ZrS₃ (a) and TiS₃ (b) nanomaterials.

In the case of TiS₃, 100% sterility of seedlings was noted at all analyzed concentrations above 0.75 µg/L (figure 3b). In addition, at 1.5 and 3 µg/L of nanomaterial, 100% plant survival was observed. At 6 µg/L, the indicator was at the control level; at 15 µg/L, the survival rate decreased by 8%. As in the case of ZrS₃, the dose of 0.75 µg/L had no significant effect on microclones.
Figures 4a and 4b show the results of studying the effect of ZrS₃ and TiS₃ on the sterility and survival of red oak micro-sprouts. As can be seen from the presented diagrams, both nanomaterials turned out to be effective sterilizing agents at concentrations above 1.5 μg/L. However, if in the case of TiS₃ 100% sterility was observed at 3 - 15 μg/L, then for ZrS₃ only at 6 and 15 μg/L there were 100% sterile seedlings, and at 3 μg/L the indicator was 90%. Moreover, even at 1.5 μg/L, TiS₃ had a small protective effect - the number of sterile seedlings increased by 5%. The maximum number of surviving seedlings was noted in the variant 3 μg/L TiS₃ - 42% (figure 4b), which is 22% higher than the control value. At 1.5 μg/L TiS₃, the number of surviving seedlings increased by 12%, and at 6 μg/L, by 5%. Zirconium trisulfide had a lesser positive effect (figure 4a). At 1.5 μg/L, the index was 8% higher than the control, and at 3 μg/L, by 6. Nanomaterials at a concentration of 15 μg/L had an inhibitory effect on the development of oak seedlings - at 15 μg/L ZrS₃, the number of surviving seedlings decreased by 10%, at 15 μg/L TiS₃ - by 3%.

![Figure 4a](image1.png) ![Figure 4b](image2.png)

**Figure 4.** Sterility and survival of oak micro shoots under the influence of ZrS₃ (a) and TiS₃ (b) nanomaterials.

Figures 5a and 5b show that the addition of ZrS₃ to the cultivation medium at concentrations of 6 and 15 μg/L ensured complete sterility of pine seedlings, at 3 μg/L 90% of clones were sterile. In the case of TiS₃, 100% sterility was also observed at 3 μg/L, i.e. titanium trisulfide has great antimicrobial properties, which are manifested already at 1.5 μg/L of the substance in the medium.

The studied nanomaterials have a negligible effect on plant survival, with the exception of the maximum concentration, where suppression of viability by 20% ZrS₃ and 13% TiS₃ was observed. Also,
a TiS$_3$ dose of 3 μg/L had a significant effect on the survival of micro-seedlings - in this case, the maximum survival of seedlings was observed (+ 12%).

Analysis of the results of studying the sterility and survival of plants under the influence of zirconium and titanium trisulfides shows a higher antimicrobial effect of TiS$_3$, with a lesser inhibitory effect on the tree crops themselves.

Those, this nanomaterial can be successfully used as a sterilizing agent at the initiation stage during clonal micropropagation of woody plants, which is confirmed by previous studies on micro shoots of poplar and birch [12]. Titanium-containing nanoparticles have shown antibacterial and antifungal properties in a significant number of studies [22-25]. Anti-microbial action of zirconium trisulfide has been shown in bacteria *E. coli* [14]. Research has shown [26], antibacterial activity of zirconium dioxide against *E. coli*, while Zr (IV) complexes are active against both *E. coli* and *S. aureus* bacteria and fungal strains.

In the course of the analysis of the biomorphological parameters of the shoots at the initiation stage, it is shown (tables 1-3) the multidirectional effect of the analyzed nanomaterials on the development of seedlings.

As can be seen from table 1, during the cultivation of brittle willow in a medium containing 1.5 μg/L ZrS$_3$, the shoot height increased 1.3 times relative to the control. In the same group, an increase in the number of leaves by 1 was noted, as well as a greater number of shoots (2 versus 0 in the control). Decrease or increase in concentration had a negative effect on seedlings. The maximum inhibitory effect was observed at 15 μg/L of nanomaterial. In this case, the height of the shoots decreased by more than 4 times, and the number of leaves by 2 times. TiS$_3$ nanoribbons stimulated micro-sprouts of brittle willow at 1.5 and 3 μg/L. Shoot height increased in these variants by 0.5 and 0.7 cm, the number of leaves by 1, and the formation of additional shoots was also noted (+1). The nanomaterial doses of 0.75 and 6 μg/L had a slight suppressive effect, and at 15 μg/L, a significant decrease in the analyzed parameters was observed. The height of the shoots decreased by 2.5 times, and the number of leaves by 2 times.

**Table 1.** Biomorphological indicators of tree seedlings at the initiation stage.

| Concentration of nanomaterial, μg/L | Shoot height, cm | Number of leaves, pcs | Number of additional shoots, pcs |
|-------------------------------------|------------------|-----------------------|----------------------------------|
|                                     | Willow           | Oak                   | Pine                             | Willow | Oak | Pine |
| Control                             | 2.5              | 0.5                   | 0.4                             | 2      | 2   | 3    | 0     | 0    | 0    |
| ZrS$_3$0.75                         | 2.4              | 0.4                   | 0.3                             | 1      | 2   | 3    | 0     | 0    | 0    |
| TiS$_3$0.75                         | 2.4              | 0.6                   | 0.4                             | 2      | 2   | 3    | 0     | 0    | 0    |
| ZrS$_3$1.5                          | 3.2              | 0.3                   | 0.3                             | 3      | 2   | 3    | 2     | 0    | 0    |
| TiS$_3$1.5                          | 3.0              | 0.5                   | 0.4                             | 3      | 2   | 3    | 1     | 0    | 0    |
| ZrS$_3$3.0                          | 2.0              | 0.3                   | 0.3                             | 2      | 2   | 2    | 0     | 0    | 0    |
| TiS$_3$3.0                          | 3.2              | 0.5                   | 0.3                             | 3      | 3   | 4    | 1     | 0    | 0    |
| ZrS$_3$6.0                          | 1.1              | 0.2                   | 0.2                             | 2      | 2   | 2    | 0     | 0    | 0    |
| TiS$_3$6.0                          | 2.2              | 0.3                   | 0.3                             | 2      | 2   | 2    | 0     | 0    | 0    |
| ZrS$_3$15.0                         | 0.6              | 0.2                   | 0.2                             | 1      | 1   | 1    | 0     | 0    | 0    |
| TiS$_3$15.0                         | 1.0              | 0.3                   | 0.3                             | 1      | 2   | 2    | 0     | 0    | 0    |

When assessing the effect of ZrS$_3$ and TiS$_3$ at various concentrations on micro shoots of red oak, a dose-dependent inhibitory effect of ZrS$_3$ on the development of oak seedlings was established; stimulation of shoot growth at 0.75 μg/L - + 20% and an increase in the number of leaves by 1 at 3 μg/L. At the same time, at 6 and 15 μg/L, the shoot height decreased 1.7 times, while maintaining the number of leaves at the level of control values.

As in the case of the red oak culture, a dose-dependent inhibitory effect of ZrS$_3$ was observed for Scots pine. Thus, at 0.75 and 1.5 μg/L, while maintaining the number of leaves at the control level, the height of the shoots decreased by 20%. At 3 μg/L with a 20% decrease in plant height, the value of the
number of leaves also decreased by 1. At 6 and 15 μg/L, the shoot height decreased by 2 times, and the number of leaves by 1.5 and 2 times, respectively. Titanium trisulfide had no effect on pine micro-sprouts at doses of 0.75 and 1.5 μg/L; however, at 3 μg/L, the plant height increased by 1.25 times, and the number of leaves by 1.3 times. A further increase in the concentration of nanomaterial negatively affected the development of seedlings. Thus, at 6 and 15 μg/L, the shoot height decreased by 2 times, and the number of leaves by 1.5 and 3 times.

Thus, at the initiation stage a higher antimicrobial effect of TiS₃ was noted at concentrations above 1.5 μg/L, while for ZrS₃, a 100% suppression of the development of microorganisms was noted at doses of 6 and 15 μg/L. Both nanomaterials had a suppressive effect on the development of seedlings at a concentration of 15 μg/L; however, with a higher antimicrobial effect of TiS₃, the toxic effect on the seedlings themselves was lower than in the case of ZrS₃. The maximum stimulating effect was observed at 1.5 μg/L ZrS₃ and 3 μg/L TiS₃.

3.2.2. Proliferation stage. After obtaining normally developed microclones, they were cuttings and transplanted onto nutrient media containing nanoparticles and hormonal components.

Evaluation of the survival rate of brittle willow shoots under the influence of nanomaterials showed (figure 6) an increase in the survival rate of seedlings in all variants with nanomaterials. The maximum values (100%) were noted in the groups: ZrS₃ and TiS₃ at a concentration of 1.5 μg/L, TiS₃ 3 μg/L, ZrS₃ 1.5 μg/L with phytohormones, and TiS₃ 3 μg/L with phytohormones. In other variants with nanoparticles, the survival rate was 90%. It should be noted that in the control group and the group cultivated only with phytohormones, the indicators were at the level of 75-76%.

![Figure 6. Survival of seedlings.](image)

For the culture of red oak in general, there was a low survival rate of seedlings - 30% in the control (figure 6). The addition of hormonal components to the medium had an even more suppressive effect, the indicator in this variant was only 25%. The addition of nanomaterials into the cultivation medium had a favorable effect on the seedlings; in all cases, an increase in the indicator was observed. The maximum values were recorded at 1.5 μg/L ZrS₃ (58%) and 3 μg/L TiS₃ (63%). It must be said that the addition of hormones to the nutrient medium with nanomaterials resulted in a 5-8% decrease in survival rate compared to options with only nanoparticles. It can be assumed that in this case, a hormonal composition not suitable for oak was chosen.

As in the case of oak, for pine, low plant survival was noted in the control group (30%) and in the group grown with phytohormones (28%) (figure 6). At 1.5 μg/L ZrS₃ and TiS₃, the survival rate increased almost 2 times, by 28 and 27%, respectively. An increase in the concentration of nanomaterials to 3 μg/L reduced the survival rates - the increase to the control in these cases was 20 and 18%. The
addition of phytohormones to media with nanoparticles did not significantly affect the viability of regenerants, with the exception of the 3 μg/L TiS3 variant, where the addition of phytohormones increased by 8%.

In general, according to the experiment, a favorable effect of ZrS3 and TiS3 nanoribbons on the viability of seedlings can be noted.

In the course of evaluating the influence of ZrS3 and TiS3 on the morphometric parameters of crops, an increase in the height of shoots under the influence of nanomaterials was established (table 2).

| Concentration of nanomaterial, μg/L | Shoot height, cm | Number of leaves, pcs | Number of additional shoots, pcs |
|-------------------------------------|------------------|-----------------------|---------------------------------|
|                                     | Willow | Oak  | Pine | Willow | Oak  | Pine | Willow | Oak  | Pine |
| Control (WPM)                       | 2.5    | 1.8  | 1.0  | 3      | 4    | 5    | 0      | 0    | 0    |
| WPM+PhH                            | 2      | 1.3  | 1.3  | 4      | 3    | 6    | 2      | 1    | 1    |
| ZrS3 1.5                           | 3.2    | 2.2  | 2    | 3      | 4    | 5    | 2      | 1    | 1    |
| TiS3 1.5                           | 3.0    | 2.2  | 2.1  | 3      | 4    | 6    | 1      | 1    | 1    |
| ZrS3 3                             | 2.7    | 2.1  | 2.1  | 3      | 4    | 5    | 3      | 0    | 0    |
| TiS3 3                             | 3.5    | 2.3  | 2.1  | 4      | 4    | 5    | 2      | 0    | 0    |
| ZrS3 1.5+PhH                       | 2.6    | 2    | 1.8  | 4      | 3    | 5    | 4      | 1    | 1    |
| TiS3 1.5+PhH                       | 2.2    | 2.1  | 1.9  | 3      | 3    | 5    | 2      | 1    | 1    |
| ZrS3 3+PhH                         | 2.5    | 2.6  | 2.6  | 4      | 4    | 6    | 4      | 2    | 2    |
| TiS3 3+PhH                         | 3.1    | 2.8  | 2.7  | 4      | 3    | 6    | 3      | 2    | 2    |

In the case of willow, the maximum positive effect was observed at 3 μg/L TiS3 +1 cm relative to the control, the minimum value of the indicator was in the variants with phytohormones (-0.5 cm) and TiS3 1.5 + phytohormones (-0.3 cm, table 2). In the ZrS3 + phytohormones group, the shoot height was at the control level; in all other cases, an increase in the growth of regenerants was observed. The maximum growth of oak shoots was recorded at 3 μg/L TiS3; the minimum was also noted when phytohormones without nanoparticles were introduced into the cultivation medium. In the case of pine, the lowest plant height was observed in the control variant, the highest regenerants were grown in the TiS3 + phytohormones medium, the indicator exceeded the control one by almost 3 times.

ZrS3 and TiS3 nanoribbons had a positive effect on the development of pine leaves; for willow and oak, the number of leaves did not differ significantly from the control values.

The most important indicator at the stage of multiplication, the presence of additional shoots, increased significantly for all crops at 3 μg/L of ZrS3 and TiS3 nanoribbons with the addition of phytohormones.

According to the results of the experiment to assess the effect of ZrS3 and TiS3 on woody crops, it can be noted that nanomaterials in the studied concentrations did not have a toxic effect, and moreover, in most cases, they increased the analyzed parameters.

3.2.3. Rooting stage. At the stage of rooting, the analyzed nanomaterials activated the rhizogenesis of willow, oak, and pine (table 3). For willow micro-sprouts, the largest number of rooted plants was obtained with the combined use of nanoparticles and auxin - 90% and more, with 5% in the control variant. The addition of auxin into the medium without nanomaterials increased the number of plants with roots to 50-53%; pure ZrS3 caused root formation in 12% of regenerants, and pure TiS3 in 15%.

As can be seen from table 3, oak and pine seedlings did not take root in an environment without hormones and nanoparticles. ZrS3 and TiS3 slightly stimulated rhizogenesis; however, under the combined action of nanoribbons and auxin, the number of plants with roots increased: in oak, by 9 and 14% when ZrS3 was introduced with 0.2 mg/L and 0.4 mg/L PhH, and by 20 and 60% when TiS3 was introduced. With 0.2 mg/L and 0.4 mg/L PhH; in pine, by 12 and 72% with the addition of ZrS3 with 0.2 mg/L and 0.4 mg/L PhH and by 18 and 73% with the addition of TiS3 with 0.2 mg/L and 0.4 mg/L PhH.
Table 3. Biomorphological parameters of tree seedlings at the rooting stage.

| Concentration of nanomaterial, μg/L | Number of seedlings with roots, % | Willow | Oak | Pine |
|-------------------------------------|----------------------------------|--------|-----|------|
| Control (WPM)                       |                                  | 5      | 0   | 0    |
| WPM+PhH 0.2 mg/L                   |                                  | 50     | 0   | 10   |
| WPM+PhH 0.4 mg/L                   |                                  | 53     | 4   | 20   |
| ZrS₃ 3                             |                                  | 12     | 2   | 3    |
| TiS₃ 3                             |                                  | 15     | 8   | 1    |
| ZrS₃ 3+PhH 0.2 mg/L                |                                  | 91     | 9   | 12   |
| TiS₃ 3+PhH 0.2 mg/L                |                                  | 90     | 20  | 18   |
| ZrS₃ 3+PhH 0.4 mg/L                |                                  | 93     | 14  | 72   |
| TiS₃ 3+PhH 0.4 mg/L                |                                  | 92     | 60  | 73   |

The results obtained show that ZrS₃ and TiS₃ nanoribbons are activators of growth and rhizogenesis of tree crops. Similar results were obtained with downy birch and poplar × aspen hybrid micropropagation [12]. As with brittle willow, red oak and scots pine, TiS₃ has been shown to be effective as a sterilizing agent, growth promoter and rhizogenesis promoter. Bioaccumulation analysis did not detect the presence of TiS₃ particles in the plants. However, elemental analysis of plant homogenates showed an increase in the amount of sulfur in the samples. Taking into account the fact that titanium and zirconium trisulfides are an unstable materials in air and water, which is confirmed by the assessment of H₂S emission, it can be assumed that the released hydrogen sulfide is absorbed by plants [27]. H₂S is known to play an important role in the regulation of normal plant physiological processes, including seed germination and root morphogenesis. [28-30]. As shown in a number of works, H₂S at low concentrations plays a decisive role in various processes of the plant life cycle, such as growth, development and responses to biotic and abiotic stress. [31, 32].

In addition, the observed stimulating effects can be associated with the antimicrobial properties of the studied nanomaterials, i.e. ZrS₃ and TiS₃ are phytoprotectants. The higher antimicrobial and stimulating effect of TiS₃ is due to the presence of the titanium dioxide phase [33], known for its bactericidal [22], fungicidal [34] and phytostimulating [35] properties.

4. Summary

Thus, in the course of the study, ZrS₃ and TiS₃ were shown to be highly effective against pathogenic microorganisms at concentrations above 1.5 μg/L. The sterility of seedlings is of great importance in microclonal propagation of plants. At the initiation stage, the maximum survival rate and the best development of willow and oak seedlings were noted at 1.5 μg/L ZrS₃ and 3 μg/L TiS₃. ZrS₃ did not have a significant effect on these parameters of pine seedlings at concentrations below 6 μg/L; at the same time, the addition of 3 μg/L TiS₃, as in the case of other crops, had a favorable effect on the viability and development of shoots. Along with the positive effects of ZrS₃ and TiS₃ on micro shoots, a negative effect of nanomaterials in concentrations of 6 and 15 μg/L was also observed, and with an increase in concentration, the toxic effect increased. Both nanomaterials had a suppressive effect on the development of seedlings at a concentration of 15 μg/L; however, with a higher antimicrobial effect of TiS₃, the toxic effect on the seedlings themselves was lower than in the case of ZrS₃.

At the proliferation stage the use of ZrS₃ and TiS₃ nanoparticles favorably affected the viability and development of shoots. As in the first stage, the concentration of 1.5 μg/L was more effective for ZrS₃, and 3 μg/L for TiS₃.

At the rooting stage ZrS₃ and TiS₃ at doses of 1.5 and 3 μg/L with auxin activated rhizogenesis, significantly increasing the number of seedlings with roots in comparison with the variants where only hormones were used. Moreover, in the case of oak and pine, the seedlings did not take root in an environment without hormones and nanoparticles.
We associate the observed effects with the combined direct action of nanoribbons and influence of hydrogen sulfide and metal oxides formed during the oxidation of ZrS3 and TiS3 in an aqueous medium. At the same time, hydrogen sulfide in low concentrations can manifest itself as a low molecular weight stimulant or as an antibiotic in high concentrations. Titanium dioxide is a well-known photoactive agent that kills microorganisms by generating reactive oxygen species. However, to clarify the exact mechanisms of the effect of trisulfides of transition metals on plants and microorganisms, additional research is needed.

The results of our work can be useful in the design of new chemical agents to increase the efficiency of biotechnology for microcloning valuable tree genotypes, which will contribute to the development of forestry. But at the same time it is necessary to take into account the limitations associated with the toxicity of nanomaterials.

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