Effects of silver nanoparticles and zirconium trisulphide nanoplates on the adaptation of woody species microclones to ex vitro conditions

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Abstract. The transfer of microclones to nonsterile conditions is one of the critical stages of micropropagation. Nanoparticles and nanomaterials can significantly improve effectiveness of this technique if used as substances increasing adaptive capabilities of the propagated plants. We have studied the impact of silver nanoparticles and zirconium trisulphide nanoplates on the adaptation of microclones of white poplar × aspen hybrid, hairy birch, crack willow, red oak and scots pine upon transfer to ex vitro conditions. The performed study has revealed that foliage application of colloidal aqueous solutions of zirconium trisulphide and silver nanoparticles at 3 µg/l concentration to in vitro microclones was more effective than the reference growth regulator. The best results were observed in the experiments with red oak microclones, where the number of surviving and adapted plants increased by 50-60 %. Increase in biomorphological parameters was accompanied by improvement of photosynthetic activity. The results indicate high potential of zirconium trisulphide nanoplates and silver nanoparticles for protecting woody species microclones during their transfer to non-sterile conditions of glasshouse.

1. Introduction

Microclonal propagation in vitro is being actively used in the production of healthy planting material of woody plants. However, it needs further improvement to increase the growth rates of microclones in vitro and enhance regenerant survivability ex vitro. The transfer of in vitro propagated plantlets to non-sterile ex vitro conditions, i.e adaptation, is the most labor-consuming stage of the micropropagation technique.

A nanobiotechnological approach based on nanoparticles and nanomaterials application for controlling phytopathogenic microorganisms is an advanced way of improving the survival rate of microclonal shoots [1, 2].

It is well known that silver nanoparticles are highly effective against a whole range of bacteria [3], fungi [4] and viruses [5], including phytopathogenic ones [6, 7].

Two-dimensional nanomaterials (e.g. graphene and its derivatives [8, 9], transition metal di- and trichalcogenides [10-12], MX-enes [13, 14] and other) are super-thin plated structures; they also can affect plants positively due to their antimicrobial properties.
Production of reactive oxygen species, cation release, biomolecule damages, ATP depletion and membrane interaction are considered to be the main mechanisms of antimicrobial activities of nanomaterials [15]. At the same time, the data on the effects produced by metallic nanoparticles and two-dimensional nanomaterials on common woody species microclones are extremely limited. Thus, in the present work we elucidate the influence of zirconium trisulphide nanoplates and silver nanoparticles on the adaptation of woody species microclones to ex vitro conditions.

2. Materials and methods
In our study, we used plantlets of white poplar (Populus alba) × aspen (Populus tremula) hybrid, hairy birch (Betula pubescens), crack willow (Salix fragilis), red oak (Quercus rubra) and scots pine (Pinus sylvestris) obtained by the microcloning propagation method. In this work, we followed the conventional methods for in vitro cultivation of isolated plant organs [16]. The cuttings were harvested from outdoor-growing plants. Freshly cut explants with apical and axillary buds were used for establishing axenic culture in vitro. Microclones were grown under laboratory conditions for 3 months. Well-developed plants with 4-5 leaves and roots no shorter than 2 cm were used for replanting into the nonsterile conditions.

In the greenhouse, the microclones were cultivated for 3 weeks at 20-24 °C, 15-h photoperiod and 80-90 % relative air humidity. A fog cannon was used in the greenhouse for maintaining the necessary humidity level. The cultivation soil consisted of peat in a good phytosanitary condition and perlite at the 3:1 ratio. For the control group, the cultivation soil was treated with a fungicidal agent (25 % solution of fludioxonil, 2 ml per 5 l of water) 24 hours prior to transplanting. The medium pH was 5.5. The soil for the test groups was treated with Ag nanoparticles at 1.5, 3 and 4.5 μg/l concentrations. Every seed starter tray consisted of six 150 ml cells, 100 cells per each experimental variant.

After planting into the soil, the microclones were fertilized with aqueous dispersions containing 3 and 4.5 μg/l Ag and ZrS₃ nanoparticles. For the positive control, a reference growth regulator (2,4-epibrassinolide) at 667 μg/l was used.

Photosynthesis activity was measured by means of a fluorimetric indicator of physiological state IFSR-2 [17].

The silver nanoparticles (AgNPs) were obtained by electric explosion of conductive wire in the atmosphere of inert gases (Advanced Powder Technologies, LLC, Tomsk, Russia). The mean particle size, according to the data of dispersion composition analysis carried out on a Zetasizer Nano ZS (Malvern, GB) and transmission electron microscopy, was 30-60 nm (figure 1a).

![Figure 1. Characterization of materials: (a) AgNPs mean size; (b) ZrS₃ sample micrograph.](image)

3. Results and discussion
Figure 2 shows how various methods of the growth medium treatment affect the rate of its contamination and the microclone survival rate.
According to our observations, the control group microclones suffered from infections and root rot, no more than 50 % of control plantlets survived.

Treatment with fungicide reduced the number of infected samples by 50 % and increased the number of surviving microclones to 70 %. Treatment with 1.5 μg/l AgNPs increased the number of samples free from infection to 75 %, the number of surviving regenerants was also 75 %.

Treatment with 3 μg/l AgNPs proved to be optimal for peat-based soil preparation for microclones transplanting, as in this variant, both soil and plantlets were free from infection and the survival rate reached 89 %. Treatment with 4.5 μg/l AgNPs eliminated infection, but the survival rate decreased to 65 %; this fact may be connected with silver nanoparticles phytotoxic activity [18-20].

The assay results for nanoparticles influence on white poplar × aspen hybrid microclones ex vitro adaptation are presented in Table 1. As one can see, the highest results for surviving plantlets (80 %) was observed after the treatment with 3 μg/l ZrS₃, which exceeded the control by 20 % and exceeded the positive control (the reference growth regulator) by 10 %. The number of adapted plants in the group treated with 3 μg/l ZrS₃ exceeded the control by 15 %. The maximal number of adapted plants (70 %) was in the group treated with 3 μg/l Ag nanoparticles compared with 50 % in the control and 60 % in the group treated with 2,4-epibrassinolide. All the other parameters were similar to those in the positive control.

Table 1. Effectiveness measures for white poplar × aspen hybrid microclones in vivo adaptation.

| Variant     | Number of surviving microclones, % | Regenerant height, cm | Number of leaves, pcs | Number of wilted leaves, pcs | Number of adapted plants, % | Microclones condition on a 1 to 5 scale |
|-------------|-----------------------------------|-----------------------|-----------------------|-----------------------------|-----------------------------|------------------------------------------|
| Control     | 60.0                              | 8.0                   | 8                     | 2                           | 50.0                        | 4                                        |
| 2,4-epibrassinolide – 667 μg/l | 70.0                              | 8.5                   | 10                    | 2                           | 60.0                        | 5                                        |
| Ag – 3 μg/l | 75.0                              | 8.5                   | 10                    | 2                           | 70.0                        | 5                                        |
| ZrS₃ – 3 μg/l | 80.0                              | 8.2                   | 8                     | 4                           | 65.0                        | 5                                        |
| Ag - 4.5 μg/l | 50.0                              | 8.0                   | 7                     | 4                           | 30.0                        | 4                                        |
| ZrS₃ – 4.5 μg/l | 52.0                              | 7.2                   | 7                     | 4                           | 36.0                        | 3                                        |
Figure 3. Photosynthetic activity of white poplar × aspen hybrid microclones.

Increased concentrations of nanoparticles significantly reduced the studied parameters. The number of surviving microclones decreased by 18-20% compared with the control, the number of wilted leaves doubled, and the number of adapted plants decreased by 20% in the nanosilver variant and by 14% in the 4.5 μg/l ZrS3 variant.

Photosynthetic activity study revealed some decrease in the parameter in the group treated with 3 μg/l Ag (figure 3), though the biomorphological analysis revealed the maximal values in this group (Table 1).

The plants treated with ZrS3 displayed a higher photosynthetic activity rate than that in the control; this indicates stimulating activity of zirconium trisulphide.

In case of hairy birch, study of the nanoparticle solutions treatment revealed that Ag nanoparticles and ZrS3 nanoplates at 3 μg/l had the maximal effect on the microclones (Table 2).

Table 2. Effectiveness measures for hairy birch microclones in vivo adaptation.

| Variant                  | Number of surviving microclones, % | Regenerant height, cm | Number of leaves, pcs | Number of wilted leaves, pcs | Number of adapted plants, % | Microclones condition on a 1 to 5 scale |
|--------------------------|-----------------------------------|-----------------------|-----------------------|------------------------------|-----------------------------|---------------------------------------|
| Control                  | 65.0                              | 4.0                   | 5                     | 4                            | 50.0                        | 4                                     |
| 2,4-epibrassinolide – 667 μg/l | 70.0                              | 4.5                   | 6                     | 2                            | 60.0                        | 5                                     |
| Ag – 3 μg/l              | 77.0                              | 5.5                   | 6                     | 2                            | 72.0                        | 5                                     |
| ZrS3 – 3 μg/l            | 85.0                              | 5.0                   | 6                     | 2                            | 65.0                        | 5                                     |
| Ag – 4.5 μg/l            | 70.0                              | 3.5                   | 6                     | 4                            | 30.0                        | 4                                     |
| ZrS3 – 4.5 μg/l          | 50.0                              | 3.7                   | 5                     | 4                            | 34.0                        | 3                                     |

Nanosilver at 3 μg/l increased the number of surviving microclones by 12%, while the same concentration of ZrS3 increased the number of surviving microclones by 20%. The maximal plant height (5.5 cm) was registered in the group treated with 3 μg/l of Ag, compared with 4 cm in the control. The same concentration of both nanoparticles twice reduced the number of wilted leaves and increased the number of adapted plants by 22% in the Ag variant and by 15% in the ZrS3. It should be noted that the reference stimulator effectiveness was below that of the nanoparticles at 3 μg/l.

Higher concentrations of nanomaterials had adverse effects on hairy birch microclones. Though in the group treated with 4.5 μg/l Ag the survival rate was slightly increased – 70% compared with 65% in the control, all the other parameters were below the control. ZrS3 solution at 4.5 μg/l had the strongest adverse effect as the number of surviving plants decreased by 15%, the number of wilted leaves doubled and the general condition of the plants was ranked at 2 points, while the control plants were ranked at 4.

Photosynthetic activity study of the hairy birch microclones treated with 3 μg/l of Ag and ZrS3 nanoparticles (figure 4) revealed that Ag nanoparticles solution produced the strongest effect.
Figure 4. Photosynthetic activity of hairy birch microclones.

In case of crack willow microclones treatment with nanopreparations, the best survival rate results (100 %) were observed when the plantlets were treated with 3 μg/l of zirconium trisulfide, while in the control, the survival rate was 80 % and 85 % survived in the positive control group (Table 3).

Application of ZrS$_3$ at 3 μg/l increased the number of adapted microclones by 15 %; it also increased the plantlet height and number of leaves. The maximal number of adapted microclones (72 %) was observed in the group treated with Ag at 3 μg/l; this exceeded the control by 22 %. As in the previous cases, the reference growth stimulator effectiveness was below that of the nanoparticles at 3 μg/l.

Table 3. Biomorphological parameters for crack willow microclones during in vivo adaptation.

| Variant                  | Number of surviving microclones, % | Regenerant height, cm | Number of leaves, pcs | Number of wilted leaves, pcs | Number of adapted plants, % | Number of additional shoots, pcs | Microclones condition on a 1 to 5 scale |
|--------------------------|-----------------------------------|-----------------------|-----------------------|-----------------------------|-----------------------------|-------------------------------|--------------------------------------|
| Control                  | 80.0                              | 4.8                   | 5                     | 3                           | 50.0                        | 0                             | 4                                    |
| 2,4-epibrassinolide – 667 μg/l | 85.0                              | 5.0                   | 6                     | 2                           | 60.0                        | 0                             | 5                                    |
| Ag – 3 μg/l              | 87.0                              | 5.3                   | 6                     | 2                           | 72.0                        | 0                             | 5                                    |
| ZrS$_3$ – 3 μg/l         | 100.0                             | 5.8                   | 6                     | 2                           | 65.0                        | 0                             | 5                                    |
| Ag – 4.5 μg/l            | 80.0                              | 4.9                   | 6                     | 3                           | 30.0                        | 0                             | 4                                    |
| ZrS$_3$ – 4.5 μg/l       | 73.0                              | 2.5                   | 5                     | 3                           | 34.0                        | 0                             | 3                                    |

In the 4.5 μg/l Ag group, the studied parameters were, as a whole, similar to those in the control, except the number of adapted plants which was 30 % in the treated group and 50 % in the control.

Treatment with 4.5 μg/l ZrS$_3$ solution had an adverse effect on the general state of the plants – the number of surviving microclones decreased by 7 %, the height decreased almost by half, only 34 % of the plantlets adapted to ex vitro conditions. The general state of the plants was ranked at 3.

Photosynthesis study of clones treated with 3 μg/l of ZrS$_3$ (figure 5) revealed a dramatic decrease in the parameter. Treatment with 3 μg/l AgNPs slightly increased activity of the photosynthetic system.

For red oak microclones adaptation, the studied nanoparticle solutions proved to be more effective than the reference growth regulator (Table 4). The best results were produced by 3 μg/l solutions of Ag and ZrS$_3$. The number of surviving microclones in these cases was 80 % and 90 %, respectively; this is by 50 % and 60 % higher than the control. Plant height after 3 weeks of cultivation measured 4.2 cm and 4.8 cm, while the control plants were 3.5 cm high. The number of fully adapted plantlets was 50 % and 70 %, respectively, while in the control group only 10 % of all the plants adapted to ex vitro conditions by the end of the 3-week period. The general state of the plants was ranked at 5.
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Figure 5. Photosynthetic activity of crack willow microclones.

Table 4. Biomorphological parameters for red oak microclones during in vivo adaptation.

| Variant                | Number of surviving microclones, % | Regenerant height, cm | Number of leaves, pcs | Number of wilted leaves, pcs | Number of adapted plants, % | Number of additional shoots, pcs | Microclones condition on a 1 to 5 scale |
|------------------------|-----------------------------------|-----------------------|-----------------------|-----------------------------|------------------------------|-----------------------------------|----------------------------------------|
| Control                | 30.0                              | 3.5                   | 4                     | 2                           | 10.0                         | 0                                 | 3                                      |
| 2,4-epibrassinolide – 667 μg/l | 45.0                              | 3.9                   | 6                     | 2                           | 20.0                         | 0                                 | 3                                      |
| Ag – 3 μg/l            | 80.0                              | 4.2                   | 5                     | 1                           | 50.0                         | 0                                 | 5                                      |
| ZrS3 – 3 μg/l          | 90.0                              | 4.8                   | 5                     | 1                           | 70.0                         | 0                                 | 5                                      |
| Ag - 4.5 μg/l          | 70.0                              | 3.0                   | 4                     | 2                           | 30.0                         | 0                                 | 4                                      |
| ZrS3 – 4.5 μg/l        | 50.0                              | 2.7                   | 2                     | 3                           | 50.0                         | 0                                 | 4                                      |

A photosynthetic activity study of the red oak microclones treated with 3 μg/l of both types of nanoparticles (figure 6) revealed a pronounced positive effect on the plant photosynthetic system. The maximal values exceeding the control almost by 1.5 times were observed in the group treated with 3 μg/l of ZrS3.

Figure 6. Photosynthetic activity of red oak microclones.

Application of 3 μg/l of ZrS3 solution to scots pine produced the best results – the survival rate was 10 % higher than that in the control, the mean plant height measured at 4.3 cm (3 cm in the control), and the plantlets developed 12 leaves as compared to 8 leaves in the control (Table 5). One should note that only in the scots pine case, in the group treated with 3 μg/l of ZrS3 solution, the number of adapted
plantlets was equal to the number of surviving clones after 3 weeks of cultivation. Treatments with 2,4-epibrassinolide and Ag 3 µg/l had a negligible effect on pine microclones.

ZrS₃ at the concentration of 3 µg/l, as in the previous cases, had an adverse effect on scots pine clones – the survival rate halved, the number of adapted plantlets decreased by 10 %, the general state of the plants was ranked at 3 points out of 5.

### Table 5. Biomorphological parameters for scots pine microclones during in vivo adaptation

| Variant                      | Number of surviving microclones, % | Regenerant height, cm | Number of leaves, pcs | Number of wilted leaves, pcs | Number of adapted plants, % | Number of additional shoots, pcs | Microclones condition on a 1 to 5 scale |
|------------------------------|------------------------------------|------------------------|-----------------------|-------------------------------|-----------------------------|----------------------------------|----------------------------------------|
| Control                      | 60.0                               | 3.0                    | 8                     | 0                             | 60.0                        | 0                                | 5                                       |
| 2,4-epibrassinolide - 667 µg/l | 60.0                               | 3.5                    | 10                    | 0                             | 60.0                        | 0                                | 5                                       |
| Ag – 3 µg/l                  | 70.0                               | 4.3                    | 12                    | 0                             | 70.0                        | 0                                | 5                                       |
| ZrS₃ – 3 µg/l                | 50.0                               | 3.0                    | 10                    | 0                             | 50.0                        | 0                                | 4                                       |
| ZrS₃ – 4.5 µg/l              | 30.0                               | 3.2                    | 8                     | 0                             | 50.0                        | 0                                | 3                                       |

A photosynthetic activity study of scots pine microclones (figure 7) revealed a positive effect of the nanoparticle treatments.

![Figure 7. Photosynthetic activity of scots pine microclones.](image)

The maximal values were reached upon treatment with ZrS₃ solution at 3 µg/l, when the measured parameter exceeded the control by 2.5 times.

### 4. Conclusion

Thus, this study has revealed that in greenhouse conditions, leaf treatment of microclones with 3 µg/l colloidal solutions of ZrS₃ and silver nanoparticles is more effective than the studied reference growth regulator. The best results were obtained for red oak microclones – the number of surviving and adapted plantlets increased by 50-60 %. An increase in biomorphological parameters was accompanied by the improved photosynthetic activity.

A positive impact of nanosilver on plants has been revealed in a number of papers, in particular, they can stimulate growth of Panicum virgatum, Phytolacca Americana [21], Brassica juncea [22], Phaseolus vulgaris and Zea mays [23]. Aleksandrowicz-Trzcinska et al. have shown that use of AgNPs leads to an increase in dry masses of roots mycorrhizal colonisation of Scots pine (*Pinus sylvestris* L.) at doses of both 5 and 50 ppm [24]. Besides, some research works have revealed that silver nanoparticles increase quantum efficiency of photosynthesis [25].

We could not discover any papers studying the influence of ZrS₃ nanoparticles on plants. Nevertheless, based on our previous studies [26, 27] it can be assumed that nanomaterials with high...
aspect ratio (e.g., nanotubes, nanowires, nanoneedles) and ZrS$_3$ nanoplates can be considered as such, can pierce plant tissues forming additional water channels. It is well known that water nutrition is one of the major factors improving the plant ability to resist stress, which is especially important at the early stages of the plant development [28].

The obtained results indicate that silver nanoparticles and ZrS$_3$ nanoplates can be successfully used for plant protection and during the adaptation of woody species microclones when transferred to nonsterile ex vitro conditions. Further studies are required in order to reveal the survival rates of the treated plants in the natural conditions and to assess nanoparticle bioaccumulation in the plant tissues.

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References
[1] Duhan J S, Kumar R, Kumar N, Kaur P, Nehra K and Duhan S 2017 Nanotechnology: The new perspective in precision agriculture. Biotechnol Rep (Amst) 15 11 doi: 10.1016/j.btre.2017.03.002
[2] Iavicoli I, Leso V, Beezhold D H and Shvedova A A 2017 Nanotechnology in agriculture: Opportunities, toxicological implications, and occupational risks. Toxicol Appl Pharmacol. 329 96 doi:10.1016/j.taap.2017.05.025
[3] Rai M K, Deshmukh S D, Ingle A P and Gade A K 2012 Silver nanoparticles: The powerful nanoweapon against multidrug-resistant bacteria. J Appl Microbiol 112(5) 841 doi: 10.1111/j.1365-2672.2012.05253
[4] Xue B, He D, Gao S, Wang D, Yokoyama K and Wang L 2016 Biosynthesis of silver nanoparticles by the fungus Arthroderma fulvum and its antifungal activity against genera of Candida, Aspergillus and Fusarium. Int J Nanomed 11 1899 doi: 10.2147/ijn.S98339
[5] Ardestani M S et al. 2015 Nanosilver based anionic linear globular dendrimer with a special significant antiretroviral activity. J Mater Sci Mater Med 26(5) 179 doi: 10.1007/s10856-015-5510-7
[6] Zakharova O V et al. 2017 Sodium tallow amphopolycarboxyglycinate-stabilized silver nanoparticles suppress early and late blight of solanum lycopersicum and stimulate the growth of tomato plants. BioNanoScience 7(4) 692 doi: 10.1007/s12668-017-0406-2
[7] Koduru J R, Kailasa S K, Bhamore J R, Kim K H, Dutta T and Vellingiri K 2018 Phytochemical-assisted synthetic approaches for silver nanoparticles antimicrobial applications: A review. Adv Colloid Interface Sci 256(14) 326 doi.org/10.1016/j.cis.2018.03.001
[8] Akhavan O and Ghaderi E 2010 Toxicity of graphene and graphene oxide nanowalls against bacteria. ACS Nano 4(10) 5731 doi.org/10.1021/nn101390x
[9] Chen J, Peng H, Wang X, Shao F, Yuan Z and Han H 2014 Graphene oxide exhibits broad spectrum antimicrobial activity against bacterial phytopathogens and fungal conidia by intertwining and membrane perturbation. Nanoscale 6(3) 1879
[10] Yang X, Li J, Liang T, Ma C, Zhang Y, Chen H, Hanagata N, Su H, Xu M 2014 Antibacterial activity of two-dimensional MoS$_2$ sheets. Nanoscale. 6 I(17) 10126 doi: 10.1039/c3nr04941h
[11] Dervin S, Dionysiou D D and Pillai S C 2016 2D nanostructures for water purification: graphene and beyond. Nanoscale 8 15115 doi:10.1039/C6NR04508A
[12] Wang Z and Mi B 2017 Environmental Applications of 2D Molybdenum Disulfide (MoS$_2$) Nanosheets Environ Sci Technol 51(15) 8229 doi: 10.1021/acs.est.7b01466.
[13] Rasool K, Helal M, Ali A, Ren C E, Gogotsi Y and Mahmoud K A 2016 Antibacterial Activity of Ti$_3$C$_2$Tx MXene. ACS Nano 10 I(3) 3674 doi:10.1021/acsnano.6b00181
[14] Rasool K., Mahmoud K A, Johnson D J, Helal M, Berdiyorov G R and Gogotsi Y 2017 Efficient Antibacterial Membrane based on Two-Dimensional Ti$_3$C$_2$Tx (MXene) Nanosheets. Sci Rep 7(1) 1598 doi: 10.1038/s41598-017-01714-3
[15] Slavin Y N, Asnis J, Häfeli U O and Bach H 2017 Metal nanoparticles: understanding the mechanisms behind antibacterial activity. *J. Nanobiotechnology* **15**(1) 65 doi: 10.1186/s12951-017-0308-z

[16] Bhujwani S S and Razdan M K 1996 Plant Tissue Culture: Theory and Practice (Revised ed.). *Elsevier Science, Netherlands* vol 5

[17] Genty B, Briantais J M and Baker N R 1989 The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim Biophys Acta* **990** 87 doi.org/10.1016/S0304-4165(89)80016-9

[18] Yan A and Chen Z 2019 Impacts of Silver Nanoparticles on Plants: A Focus on the Phytotoxicity and Underlying Mechanism. *Int J Mol Sci* **20**(5) 1003 doi: 10.3390/ijms20051003

[19] Vinkovic T, Novák O, Strnad M, Gessler W, Jurašin D D, Paradiković N and Vrček I V, 2017 Cytokinin response in pepper plants (Capsicum annuum L.) exposed to silver nanoparticles. *Environ* **156**(10) doi: 10.1016/j.envres.2017.03.015

[20] Kanchan V, Neha U, Jaspreet S, Sheo M P, Devendra K C, Durgesh K T and Shivesh S 2017 Differentiation. Phytotoxic Impact of Plant Mediated Silver Nanoparticles (AgNPs) and Silver Nitrate (AgNO₃) on Brassica sp. *Front Plant Sci* **8** 1501 doi.org/10.3389/fpls.2017.01501

[21] Yin L, Colman B P, McGill B M, Wright J P and Bernhardt E S 2012 Effects of silver nanoparticle exposure on germination and early growth of eleven wetland plants. *PLoS One* **7**(10) 47674 doi: 10.1371/journal.pone.0047674

[22] Sharma P, Bhatt D, Zaidi M G, Saradhi P P, Khanna P K and Arora S 2012 Silver nanoparticle-mediated enhancement in growth and antioxidant status of Brassica juncea. *Appl Biochem Biotechnol* **167**(8) 2225 doi: 10.1007/s12010-012-9759-8

[23] Salama H M H 2012 Effects of silver nanoparticles in some crop plants, common bean (Phaseolus vulgaris L.) and corn (Zea mays L.). *Int Res J Biotech* **3**(10) 190

[24] Aleksandrowicz-Trzcinska M, Szaniawski A, Studnicki M, Bederska-Blaszczyk M, Olchowik J and Urban A 2018 The effect of silver and copper nanoparticles on the growth and mycorrhizal colonisation of Scots pine (Pinus sylvestris L.) in a container nursery experiment. *iForest* **11**(5) 690 doi: 10.3832/ifor2855-011

[25] Zainab M A and Amjad A 2015 Effect of Silver Nanoparticles on Seed Germination of Crop Plants. *Int. J. of Nuclear and Quantum Eng.* **9** 6 doi:10.24297/jaa.v4i1.4295

[26] Smirnova E, Gusev A, Zaitseva O, Sheina O, Tkachev A, Kuznetsova E, Lazareva E, Onishchenko G, Feofanov A and Kirpichnikov M 2012 Uptake and accumulation of multiwalled carbon nanotubes change the morphometric and biochemical characteristics of Onobrychis arenaria seedlings. *Front Chem. Sci. Eng.* **6**(2) 132 doi: 10.1007/s11705-012-1290-5

[27] Smirnova E A, Gusev A A, Zaitseva O N, Lazareva E M, Onishchenko G E, Kuznetsova E V, Tkachev A G, Feofanov A V, and Kirpichnikov M P 2011 Multi-walled Carbon Nanotubes Penetrate into Plant Cells and Affect the Growth of Onobrychis arenaria Seedlings. *Acta Naturae* **3**(1) 99 doi: 10.32607/20758251-2011-3-1-99-106

[28] Salisbury F B and Ross C W 1992 *Plant physiology* (Calif.: Wadsworth Pub. Co., Wadsworth biology series) p. 682