Environmental aspects of the use of ultrafine particles SiO$_2$ (as exemplified by Solanum tuberosum)

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Abstract. The interest in the use of ultrafine particles in crop production and agricultural practice is associated with the unique properties of ultrafine particles (UFP). At the same time, it is obvious that artificial nanoobjects can have toxic properties. Therefore, for further development of nanotechnologies, a clearer understanding of both the properties of nanomaterials themselves and the mechanisms of their interaction is needed. The article presents studies on the effect of ultrafine particles of silicon dioxide on plants Solanum tuberosum. According to the results of research nano-silica at a concentration of 0.21 and 0.36 g/kg had a statistically significant effect on the number of cell integrity (a decrease to 17.1 % was observed). It was revealed that after processing potato tubers with UFP silicon oxide at a concentrations of 0.09 and 0.18 g/kg, they had a significant influence on the activity of peroxidase. The results of the studies revealed the toxicity of UFP SiO$_2$ at a maximum dilution of 0.36 g / kg, which confirmed a decrease in the intensity of the luminosity peaks of DNA a temporally calculated linear profile. Therefore, assessing the impact of SiO$_2$ use is of great importance for understanding the long-term environmental impact of UFP.

1. Introduction

Today, ultrafine particles are becoming an important product of innovative science and technology with broad prospects for use both in industry and in agriculture.

Due to the enormous specific surface of the UFP, they can be effectively used in microdoses. So, for example, for pre-sowing treatment of 1 ton of seeds, several milligrams of nanopowder are used [1].

The effect on plant seeds with dispersed systems containing nanoparticles significantly increases the germination energy, growth activity of seedlings, and plant survival, which positively affects all elements of the crop structure [2, 3].

Presowing soaking seeds in a suspension of ultrafine particles affects the respiration and oxidative phosphorylation in mitochondria from cells of roots of the seedling and the photochemical activity of the chloroplasts of leaves during different phases of ontogeny.

Thus, in literature there are many experiments to assess the yield and biometric parameters of plants using the UFP as an example [4, 5].

However, the potential environmental impacts and plant biota are not well understood. In this connection it is necessary to develop a suitable methodology for the identification of interactions...
between UFP with cellular components in order to achieve a better understanding and definition of the principles of safe use [6, 7].

**Purpose of the study.** In this study, we used ultrafine particles of silicon dioxide with the diameter of 30 nm to analyze their effect on peroxidase activity, cell viability, and the integrity of DNA molecules of the *Solanum tuberosum* plant.

**2. Materials and methods**

**2.1 Chemical Substances and Substrates**

In the studies, we used silicon oxide nanoparticles (NP SiO$_2$) with a size of 30.7 ± 0.3 and a z-potential of 27 ± 0.12 mV, obtained by plasmochemical synthesis of LLC "Advanced Powder Technologies" (Russia, Tomsk).

**2.2 Test-organisms**

Potato tubers (*Solanum tuberosum*) of the variety "Tarasov" were used as the object of the study. Seed material was obtained by propagation on a virus-free basis (in vitro) and provided to the Federal State Budgetary Scientific Institution of the Ural Federal Agrarian Research Center, Ural Branch of the Russian Academy of Sciences (Chelyabinsk). All laboratory analyzes were carried out in accredited laboratory of the Federal Scientific Center for Biological Systems and Agrotechnologies of the Russian Academy of Sciences (Orenburg).

Testing the biological activity of SiO$_2$ NPs on potato tubers was carried out using examples of five concentrations of silicon dioxide (0.03, 0.09, 0.18, 0.21 and 0.36 g/kg potatoes) in triplicate. The suspension of nanoparticles of SiO$_2$ was prepared according to TU 931800-4270760-96.

The ultrafine particles were dispersed in the ultrafine bath "Sapphire TTZ" with a frequency of 35 kHz for 30 minutes.

The activity of peroxidase was determined by the method of Boyarkin, guaiacol used as a substrate. The increase in optical density at 580 nm was measured in a reaction mixture consisting of 0.5 ml of 0.1 M of citrate-phosphate buffer (pH was 6.2 and 5.4), 0.5 ml of 0.3 % hydrogen peroxide and 0.5 ml (0.035, 0.04, 0.045, 0.5, 0.055 %) of guaiacol.

Analysis of cell viability (CV) by changing the enzymatic activity of reductases was carried out according to the manufacturer's protocol (WST-8 patent No.2.251.850, Canada) using the highly sensitive test "Cell counting kit-8 (CKK-8)" ("Sigma-Aldrich", USA) based on the use of the water-soluble tetrazolium salt (WST-8).

DNA isolation: 1000 mg of the roots were divided into aliquots of 250 mg, frozen with liquid nitrogen for 5 min, 450 µl of TSB buffer was added to the samples. Samples were shaken using the homogenizer «TissueLyser LT» in the following mode: frequency ~ 50 Hz for 30 seconds. Then it was heated at 95 °C for 10 minutes to inactivate DNAase (DNA technology thermostat). 10 % SDS in the amount of 50 µl (final conc. – 1 %), 2 µl of proteinase K were added. MICROSPIN FV-2400 was shaken for 5 seconds and incubated for 30 min at 60 °C. The phenol-chloroform mixture (1:1) was added in an equal volume, i.e. 500 µl and centrifuged (14,500 on / min, 5 min) (Microspin 12). An aqueous phase of 400 µl was taken; an equal volume of chloroform-isooamyl alcohol was added. Cenrifuged (14500 on/min, 5 min). An aqueous phase of 350 µl was taken, 40 µl of 10M ammonium acetate (10:1) and 1000 µl of ice-cold absolute alcohol (−20 °C) were added. For precipitation, DNA was left overnight in a freezer (−20 °C). It was centrifuged at 4 C, 14,000 rpm for 30 min. The alcohol was removed and 400 µl of 80 % cold ethyl alcohol was added, centrifuged at 4 °C, 14000 rev/min for 10 min, then 30-501 MQ was added.

**2.3 Statistical processing**

The results were processed by methods of variation statistics using the Microsoft Office software suite by means of the “Excel” program (“Microsoft”, USA) with data processing in the “Statistics 10.0” program (Stat Soft Inc., USA). The results of P≤0.05 were considered reliable.
3. Discussion of the results
The use of peroxidase as a marker of stress allows more fully characterizing the protective moves for the diagnosis of resistance to stress factors. During the treatment of potato tubers with nanoparticles NPs SiO$_2$ at a concentration of 0.09 and 0.18 g/kg, peroxidase activity increased twofold in comparison with the control and amounted to 532.9 and 513.2 conventional units / g of wet weight, respectively. In the remaining variants, it varied from 224.8 to 461.8. The enzyme activity is expressed in conventional units / g of fresh weight.

Roohizadeh et al. (2015) [8] showed that silicon dioxide at a concentration of 1.5 and 3 mM significantly increased peroxidase activity in Vicia faba L plants, which, in its turn, leads to less damage, unlike AFK, and protects plants from stress.

![Figure 1](image1.png)

**Figure 1.** The activity of peroxidase germs *Solánum tuberósum* after the exposure to nanoparticles NPs SiO$_2$ expressed in conventional units/g of fresh weight with significant values of P <0.05.

Cell viability (CV) was studied by the yield of formazan from the water-soluble tetrazolium salt (WST). The presence of SiO$_2$ in the environment in concentrations of 0.21 and 0.36 g/kg resulted in the reduction of formazan for 24 hours incubation (up to 17.1 % relative to the control), while the remaining concentrations were (0.03, 0.09, 0.18 g/kg). The increase in reductase activity was not significant (up to 5.2 %).

![Figure 2](image2.png)

**Figure 2.** Cell viability of *Solánum tuberósum* roots after exposure to NP SiO$_2$. 

A DNA molecule is the genetic material of a cell and any damage to the DNA will cause changes in the encoded proteins, which can lead to malfunction or complete inactivation of the encoded proteins. Increased DNA degradation was observed in plants exposed to environmental stresses such as metallic NPs and carbon nanomaterials [9].

An analysis of the electrophoretic mobility of the fraction of DNA extracted from the roots of *Solánum tuberósum* after exposure to nanosilica did not show pronounced molecular degradation. The peculiarity of the effect of NPs SiO$_2$ was only a directly proportional decrease in the total DNA content visualized on the electrophoregram in the form of a decrease in fluorescence intensity to 86% (Fig. 3).

![Electrophoregram of DNA from *Solánum tuberósum* roots after incubation with various concentrations of NPs SiO$_2$ in concentrations from 0.03 to 0.36 g/kg for 72 hours: 0 – control (distilled water); M – DNA electrophoretic mobility marker (1 Kb); the graph shows the values of % of the total fluorescence area of DNA fragments calculated in the ImageJ program compared to the control; reliability P<0.05.](image)

**Figure 3.** Electrophoregram of DNA from *Solánum tuberósum* roots after incubation with various concentrations of NPs SiO$_2$ in concentrations from 0.03 to 0.36 g/kg for 72 hours: 0 – control (distilled water); M – DNA electrophoretic mobility marker (1 Kb); the graph shows the values of % of the total fluorescence area of DNA fragments calculated in the ImageJ program compared to the control; reliability P<0.05.

A number of studies have shown that optimization of silicon nutrition increases the stability of DNA and RNA molecules [10]. It has also been suggested that a silicon atom as an analogue of phosphorus can integrate into nucleic acids and thus increase their resistance to adverse conditions [11]. Recently, it has been experimentally shown that silicon has the ability to influence plant resistance to any abiogenic and biogenic stresses at the level of the plant genetic apparatus [12]. Subsequently, we decided to evaluate and analyze the consequences of direct contact of UFP with DNA of the plant *Solánum tuberósum* after 72 hours. The results of the studies have shown that the degradation of the molecules is confirmed by temporally calculated linear profiles (Fig. 4). This clearly shows a decrease in the intensity of the luminosity peaks of DNA in the case of maximum dilution – 0.36 g/kg, and the increasing peaks, approaching the control variant – in the case of concentrations of 0.18 and 0.03 g/kg.
Figure 4. Electrophoregram of Linear profiles of the bands of DNA isolated from the roots of Solanum tuberosum after exposure to NPs SiO$_2$ at concentrations of 0.36, 0.18, 0.03 g/kg for 72 hours (profiles obtained in the ImageJ program): the graph shows the luminescence intensity of DNA bands (axis abscissa) on each pixel of an electrophoregram track (ordinate axis).

A similar result was observed in the work [14] showing that at doses of 100, 300, 500, and 700 kg/ha of amorphous silica, the DNA content decreased to 112.3 ± 0.8; 119.0 ± 0.6; 120.3 ± 0.7 and 124.1 ± 0.3, respectively.

4. Conclusion
Summarizing the results on the biological activity of silicon dioxide nanoparticles in relation to the model plant Solanum tuberosum, we can conclude that SiO$_2$ exhibited the highest peroxidase activity at concentrations of 0.09 and 0.18 g/kg.

It was established that a decrease in the viability of root cells was observed to a greater extent at a concentration of 0.21 and 0.36 g/kg and caused inhibition of formazan to 17.1 % relative to control for 24 h of incubation.

According to the results of the research, linear profiles of the samples indicate the absence of visible DNA degradation.

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