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## EVALUATION OF THE EFFECT OF SILICON OXIDE NANOPARTICLES OF DIFFERENT ORIGIN ON THE PRODUCTION OF SOME PRIMARY AND SECONDARY METABOLITES IN PLANTS OF THE FAMILIES FABACEAE, POACEAE\*

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Scientists in different countries are conducting research on assessing a possibility of using silicon nanoparticles in various fields: medicine, pharmacy, medicinal crop production, etc. The work presents results of evaluating an influence of nanoparticles of different origin on production of primary and secondary metabolites. Nanoparticles, obtained from monomineral sand of natural origin, have been shown to increase production of primary and secondary metabolites in plants of the *Poaceae* family, as well as a content of a sum of chlorophylls, carotenoids, flavonoids, hydroxycinnamic acids,  $\alpha$ - and  $\beta$ -chlorophylls and a majority of amino acids contained in leaves of *Avena sativa* (*Poaceae*) and *Triticum aestivum* (*Poaceae*).

Nanoparticles, obtained from synthetic quartz glass, influence production of only primary metabolites, an increase in a content of silicon and some amino acids in leaves and stems of *Triticum aestivum* (*Poaceae*). An effect of nanoparticles of different origin on a dynamics of accumulating sum of flavonoids and hydroxycinnamic acids by the example of the *Fabaceae* family has shown their increase in samples, collected at the beginning of a vegetation season. Morpho-structural analysis of nanoparticles of different origin has revealed differences in a morphology of particles, polymerization of silicon-oxygen chains, which probably explains the presence of distinctive features of a structure and peculiarities of their influence on production of metabolites in plants.

*Keywords:* metabolites, nanoparticles, silicon dioxide modifications, *Poaceae*, *Fabaceae*.

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### Introduction

In the last few years, publications about an influence of various nanoparticles (NP) on growth processes have appeared in the literature [1–7]. At the same time, many authors [7–12] note prospects of using NP as biostimulators of plant growth in medicinal crop production and plant biotechnology (a culture of plant cells and tissues *in vitro*).

Silica nanoparticles (SiO<sub>2</sub> NP) have a high commercial appeal; they have a wide range of applications in different fields, starting from high-tech industry and ending with use in agriculture, environmental “green”

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technologies, pharmacy, medicine, medicinal crop production [13, 14]. SiO<sub>2</sub> NP can be used to deliver active ingredients, acting as bio stimulants, to plants [15, 16]. Molecular studies have revealed a positive effect of SiO<sub>2</sub> NP on biochemical physiological processes in a plant, but a detailed understanding of changes in genes [17], proteins, primary and secondary metabolites of the SiO<sub>2</sub> NP influence is still absent. However, all researchers note that the introduction/presence of SiO<sub>2</sub> NP in plants provides advantages under various abiotic and biotic stresses, increasing biochemical and physiological processes, transcriptomic and metabolic reactions [18–20].

Studying an opportunity of using SiO<sub>2</sub> NP in medicinal crop production is an urgent task. Previously, in our studies about the influence of SiO<sub>2</sub> NP on medicinal plants, an understudied fact was established that an accumulation of secondary metabolites in a plant is affected not only by morphofunctional characteristics of SiO<sub>2</sub> NP, but also by a “nature” of a source raw material [21].

The purpose of the work was to study an influence of SiO<sub>2</sub> NP of silica, obtained from silicon dioxide of different origin, on production of some primary and secondary metabolites in plants of the *Fabaceae*, *Poaceae* families.

### Experimental

When conducting the research, suspensions of silicon dioxide nano powders, obtained from samples of silicon-containing raw materials: synthetic quartz glass (hereinafter referred to as KU-1) and monomineral quartz sand (hereinafter referred to as sand), were used.

A silicon dioxide nano powder from KU-1 was obtained by laser evaporation [22], using a CO<sub>2</sub>-laser, operating in a continuous mode with a lasing power of 107 W (an experimental installation, G.K. Borekov Institute of Catalysis, SB RAS, Novosibirsk). An evaporable target was quartz glass (an optical mark was KU-1, GOST 15130-86); cylinder dimensions were 15×20 mm. A characteristic deposition rate of nanoparticles on a filter was 1.4–1.5 g/h, the evaporation temperature of quartz was about 2000 °C, and the pressure was 100 – 200 torr. The size of the obtained nanoparticles was 8–16 nm.

A quartz sand powder, whose particle size of silicon oxide was 7.5–22 nm, was obtained on the basis of an industrial electron accelerator ELV-6, intended for processing materials with a concentrated electron beam, released into the atmosphere (INP SB RAS, Novosibirsk) [23]. In an evaporation and condensation chamber (a sublimator), when steam interacts with a cold gas, steam is sharply cooled, preventing growth of particles of the obtained nano powder. The temperature of evaporating quartz in argon was 3000 °C in the air atmosphere. An electron beam at an accelerating voltage of 1.4 MeV was used as a heating element for an evaporation process of the quartz material (natural quartz sand (GOST 8736-2014)). Nano powders, obtained using the mentioned methods and equipment, are X-ray amorphous.

A solution at a concentration of  $1.5 \times 10^{-4}$  mg/l of nanoparticles in water (pH was 5.9) was prepared for making suspensions. Before treating seed material, solutions were exposed to ultrasound to avoid an agglomeration of nanoparticles (an ultrasonic bath “WiseCleanWUC”) for 15 minutes at an ultrasound frequency of 28 kHz.

Seeds, leaves, stems, roots of species of the Gramineous plant family (Gramineae) or Bluegrass (*Poaceae Barnhart*): cultivated oat (*Avena sativa L.*), soft wheat (*Triticum aestivum L.*); Legume families (*Fabaceae Lindl.* or *Leguminosae*): creeping alfalfa (*Medicago sativa L.*), were collected by the authors of the work in different phenophases: sprouting, tillering, branching, the beginning of budding. The studied raw materials were divided into organs and dried in a shade under a canopy at ambient temperature. A representative sample was taken from 25-50 individuals of each species for the analysis.

A field study was performed at a scientific and experimental hospital of SibSMU according to a field technique of the state variety testing [24].

The seed material was treated with suspensions of SiO<sub>2</sub> NP according to a standard procedure for semi-dry disinfection (10 l/t) followed by an exposure of 4-6 hours in a closed volume at  $22 \pm 1$  °C.

A content of  $\alpha$ - and  $\beta$ -chlorophylls, their sum, a sum of carotenoids were determined by spectrophotometry using a “SF-2000” spectrophotometer (Russia). A concentration of pigments was calculated based on chlorophylls and  $\beta$ -carotene [25].

A sum of hydroxycinnamic acids was determined by spectrophotometry (SF-2000, Russia) in terms of chlorogenic acid according to a procedure [25], adapted for the studied species (extraction time was 45 minutes, aliquot of a solution B was 4 ml).

A content of flavonoids was assessed in terms of quercetin according to a procedure of the State Pharmacopoeia of the Russian Federation of the 14th edition for the field horsetail grass, adapted for the studied objects [26, 27].

Morphological peculiarities of nano powders were studied using a scanning electron microscope "Tescan-Mira 3 LMU" (Czech Republic) combined with an INCA Energy350 Oxford spectrometer (Great Britain). Luminescent analysis (XRL, TL) was conducted using an original installation, manufactured on the basis of a monochromator "MDR-12" with a "BSV-2" X-ray tube from a "URS-55" apparatus with a molybdenum anticathode. The fieldwork was carried out in ultraviolet and visible regions of a spectrum; results were reflected in corresponding graphs. Luminescent characteristics were successively obtained in a radiation band of 200–800 nm.

IR-spectra were recorded using an IRPrestige-21 spectrophotometer (Shimadzu, Japan) with the Fourier transform (FTIR-8400S) in a range of 300–4000  $\text{cm}^{-1}$  at a resolution of 0.001  $\text{cm}^{-1}$  (FT-IR), using Irsolution software (Analytical Laboratory of the Department of Geology and Mineral Exploration of the Institute of Natural Resources, TPU). A studied sample weight of less than 1 mg was used, which was thoroughly mixed with a KBr powder, and a mixture was pressed in the form of a translucent tablet.

Studies by the methods of electron microscopy and X-ray structural analysis were performed using equipment of the Tomsk Regional Center for Collective Use of National Research Tomsk State University.

### **Results and Discussion**

It is known that silicon (Si) is present in plants in the form of a silicic acid  $\text{Si}(\text{OH})_4$  [28, 29]. Revealing any influence (stress factors, pathogens, treatments) manifests itself at the earliest stages of plant development.

As is known, central (primary) metabolism includes reactions and biochemical pathways that are responsible for production and energy metabolism of plant growth, development and survival. And specialized (secondary) metabolism includes processes of interaction with the environment.

This study has established the fact that treatment with suspensions of silica nanoparticles, based on the claimed powders of different origin, contributed to faster germination (vigor/viability) of seeds relative to control by 12–36%. It also allowed in creasing the growth of a number of roots by 28–45%, which looked much healthier/stronger/thicker than those of the control variants. Their growth rate and length increased by 10–66%, which together contributed to further ontogenesis of plants and allowed forming a stable phytocenosis with improved production properties, which were expressed in qualitative characteristics of raw materials.

An influence on a quality of raw materials was assessed by an effect exerted on production of some primary and secondary metabolites ( $\alpha$ - and  $\beta$ -chlorophylls, carotenoids, amino acids, hydroxycinnamic acids, flavonoids).

An influence of silicon nanoparticles from sand was manifested by a significant increase in their content in some primary and secondary metabolites. Results of their quantitative determination are shown in figures 1–4, table, which represent average values of three determinations.

The most significant increase in a content of a sum of flavonoids and hydroxycinnamic acids in leaves is observed when they are exposed to silicon nanoparticles from sand compared with KU-1 and control (fig. 1). Therefore, a content of a sum of flavonoids in oat leaves increased by  $33\pm 3\%$ ; a sum of hydroxycinnamic acids in wheat leaves increased by  $20\pm 1.9\%$ . An increase in a content of the same groups of substances under the action of KU-1 was less significant and amounted to  $21\pm 2\%$  (a sum of flavonoids) and  $14\pm 1.3\%$  (a sum of hydroxycinnamic acids), respectively.

An influence of nanoparticles on a content of silicon in leaves and stems of the studied plants was manifested in different directions (fig. 2). In leaves and stems of oats, a decrease in a silicon content by  $5\pm 0.4$  and  $14\pm 1.3\%$  was observed; in wheat, an increase in its content by  $4\pm 0.35$  and  $30\pm 2.9\%$  was noted. An effect of KU-1 on a silicon content appeared to be more significant for wheat, while a silicon content increased by  $59\pm 5.2$  and  $150\pm 14.3\%$ .

A content of pigments (a sum of chlorophylls,  $\alpha$ - and  $\beta$ -chlorophylls, a sum of carotenoids) in the studied samples increased against the background of exposure to silicon nanoparticles from sand and KU-1 (fig. 3). The most significant increase was against the background of exposure to sand nanoparticles, which amounted to 23–26% in oats and 32–37% in wheat.

An increase in a content of secondary metabolites in monocotyledonous/cereal crops against the background of exposure to nanoparticles of different nature indicates an increase in a biochemical activation of physiological processes. In future, a more detailed study requires raising an issue of a more pronounced and significant effect of sand nanoparticles on a content of secondary metabolites.

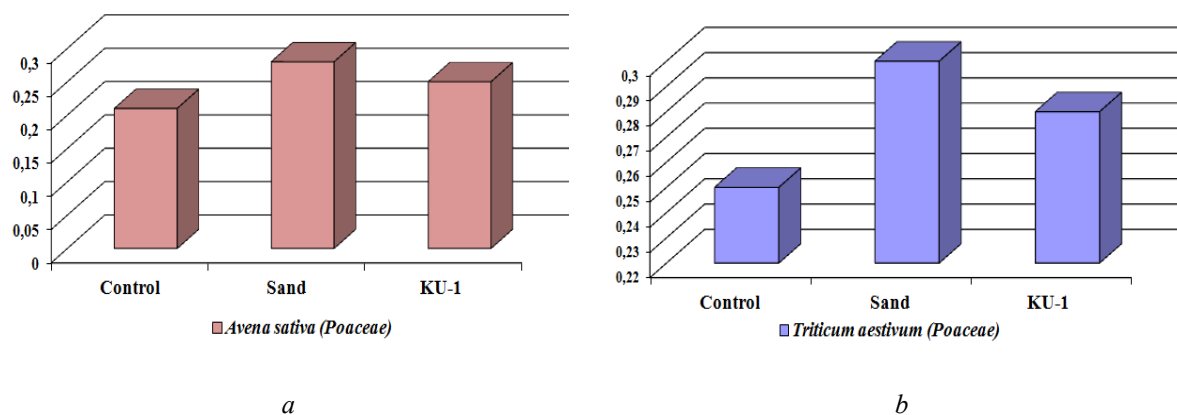


Fig. 1. Content of flavonoids (a), hydroxycinnamic acids (b) in leaves of *Avena sativa* and *Triticum aestivum* (Poaceae), % (in terms of abs.dry raw material)

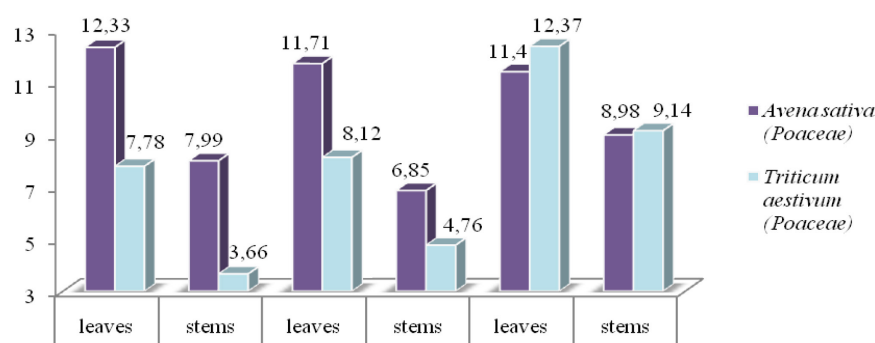


Fig. 2. Silicon content in leaves and stems of *Avena sativa* and *Triticum aestivum* (Poaceae), %

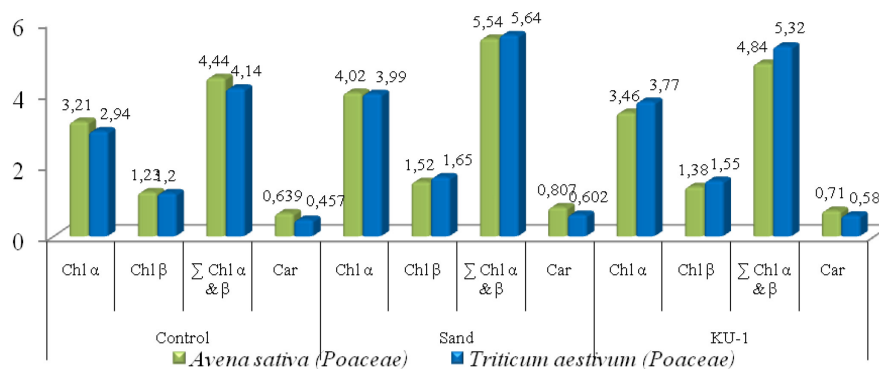


Fig. 3. Content of chlorophylls, α- and β-chlorophylls, a sum of carotenoids in samples of *Avena sativa* and *Triticum aestivum* (Poaceae), mg/g of dry weight

In most cases, an influence of silicon nanoparticles from sand and KU-1 on production of primary metabolites of oats and wheat led to an increase in a content of amino acids (table). An exception was a content of methionine, tryptophan, cystine in both crops, as well as a content of lysine, asparagine, threonine in wheat, which either did not change or changed slightly.

An increase in a content of leucine, isoleucine, phenylalanine, valine, serine, tyrosine, and methionine appeared to be significant against the background of exposure to sand and KU-1 nanoparticles. An increase in a content of leucine, tyrosine, and phenylalanine in wheat against the background of KU-1 became most significant, which amounted to 35–57%, and 58–67% against the background of sand. This metabolic shift may indicate a subtractive activity of biosynthesis of metabolites, associated with protection.

An influence of nanoparticle powders on a dynamics of accumulating flavonoids, hydroxycinnamic acids in alfalfa, a dicotyledonous plant of the *Fabaceae* family, in raw materials that were harvested from the end of May to the end of July was studied (fig. 4).

In samples that were collected in May, a content of flavonoids increased significantly when exposed to KU-1 and sand nanoparticles (13.5–15 times, respectively). In June and July, a content of flavonoids did not change when exposed to KU-1 and decreased 1.2 times when exposed to silicon nanoparticles from sand.

An effect of nanoparticle powders on a content of hydroxycinnamic acids in samples, collected in May, was more pronounced. An exposure to KU-1 led to a 127-time increase in their content and a 147-time increase when exposed to sand silicon nanoparticles. A content of hydroxycinnamic acids in alfalfa samples, collected in June, practically did not change; it increased only 1.2 times when exposed to KU-1 and decreased 1.2 times when exposed to silicon nanoparticles from sand. In samples, collected in July, against the background of KU-1, a content of hydroxycinnamic acids virtually did not change, while, when exposed to silicon sand nanoparticles, their content decreased 1.3 times.

Analysis of the obtained data about the influence on primary and secondary metabolites shows that a specificity of the effect of silicon dioxide nanoparticles of different origin is unequal. Therefore, we conducted a research on morphofunctional characteristics of powders.

Amino acids content, % (in terms of dry matter)

Amino acids	Control	SiO <sub>2</sub> NP		Control	SiO <sub>2</sub> NP	
		KU-1 (synt. quartz glass)	quartz sand		KU-1 (synt. quartz glass)	quartz sand
	<i>Triticum aestivum</i> ( <i>Poaceae</i> )			<i>Avena sativa</i> ( <i>Poaceae</i> )		
Lysine	0.39	0.49	0.53	0.35	0.68	0.74
Leucine	0.68	1.05	1.14	0.73	1.13	1.42
Isoleucine	0.35	0.47	0.53	0.41	0.38	0.42
Methionine	0.19	0.22	0.24	0.17	0.13	0.19
Cystine	0.22	0.29	0.32	0.20	0.20	0.27
Phenylalanine	0.43	0.62	0.71	0.50	0.66	0.69
Tyrosine	0.19	0.30	0.31	0.19	0.22	0.27
Threonine	0.41	0.45	0.41	0.38	0.72	0.77
Tryptophan	0.13	0.16	0.15	0.12	0.16	0.13
Valine	0.48	0.65	0.77	0.57	0.87	0.93
Asparagine	0.65	0.72	0.83	0.68	0.86	0.91
Serine	0.33	0.51	0.49	0.41	0.28	0.40

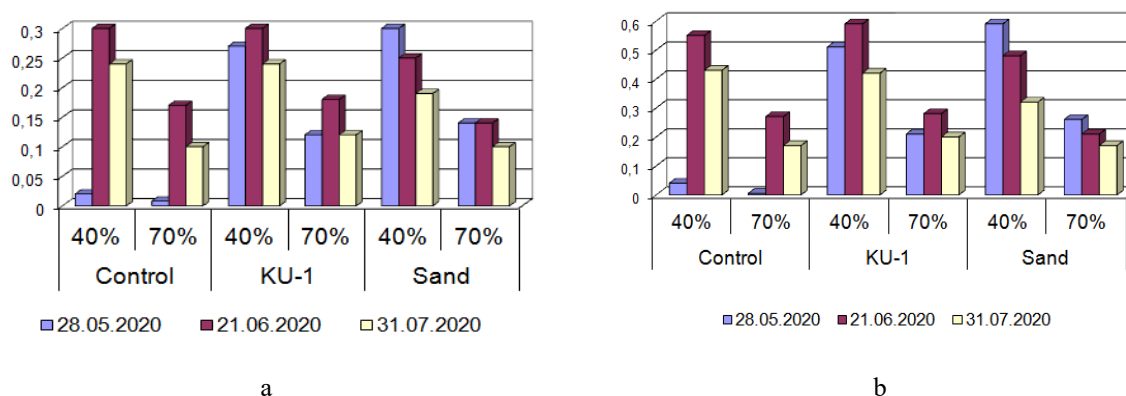


Fig. 4. Content of flavonoids (a), hydroxycinnamic acids (b) in *Medicago sativa* L (*Fabaceae*), % (in terms of abs. dry raw material)

When analyzing a morphology of nanoparticles of powders, obtained from different raw materials, a significant difference was found (fig. 3).

NP obtained from quartz glass (KU-1) are represented by particles/individuals in the form of flakes of complex configuration (fig. 1 A, A1, is presented in the electronic supplemental material), whose dimensions reach 15  $\mu\text{m}$ , which corresponds to declared characteristics (of the method of obtaining); they easily stick together and form lumpy aggregates. A sample obtained from quartz sand (sand) (fig. 1 B, B1 is presented in the electronic

supplemental material) consists/is composed of globular particles, whose dimensions are highly unstable (particles of 0.5–1  $\mu\text{m}$  are clearly distinguishable in a thin powder matrix) and differ greatly from those declared by the method (more than 15 times). The powder easily crumbles under mechanical action, forming a looser aggregate due to rounded particles/individuals.

IR-spectra of NP powders were analyzed taking into account the X-ray amorphousness of the substance [30]. In the IR spectra of silicon dioxide powders (fig. 2 is presented in the electronic supplemental material), absorption/reflection bands lie in regions associated with fluctuations in Si-O bonds. Symmetric deformation vibrations caused by Si-O-Si bending in a  $\text{SiO}_4$  tetrahedron belong to  $\sim 445\text{--}455\text{ cm}^{-1}$ . Deformation vibrations caused by a ring structure are at  $775\text{ cm}^{-1}$ .  $800\text{ cm}^{-1}$  represents deformation vibrations caused by a  $\text{SiO}_2$  network structure.  $805\text{ cm}^{-1}$  shows symmetrical deformation vibrations caused by Si-O-Si stretching between tetrahedra.  $940\text{ cm}^{-1}$  presents a line caused by vibrations of an oxygen-non forming bridge between groups of  $\text{SiO}_4$  tetrahedra or the presence of valence vibrations of a Si-OH bond. A line caused by fluctuations of an oxygen-nonforming bridge between groups of  $\text{SiO}_4$  tetrahedra or a presence of valence vibrations of a Si-Li bond belongs to  $965\text{ cm}^{-1}$ .  $1125\pm 1145\text{ cm}^{-1}$  shows deformation vibrations caused by bending a Si-O-Si structure in  $[\text{Q}_4]$ . In addition to these bands, describing vibrations inside and between tetrahedra, absorption bands, associated with vibrations in a Si-OH silanol group, a free (OH) group, and water molecules physically adsorbed into a powder composition, are present in the spectra. A peak of  $\sim 1640\text{ cm}^{-1}$  and a wide asymmetric one of  $\sim 3400\text{--}3450\text{ cm}^{-1}$  determine the presence of adsorption water. A peak of  $\sim 2900\text{ cm}^{-1}$  is caused by valence vibrations as a result of bond stretching in a free (OH) group [31].

A morphology of spectra of luminescence with the silicon dioxide powder differs significantly from spectra of natural silicon dioxide compounds (quartz, chalcedony, opal, etc.). First of all, they are distinguished by a weak luminescence intensity, which indicates chemical purity and, as a consequence, a minimum number of structural (intrinsic/impurity) defects (fig. 3 is presented in the electronic supplemental material). However, despite an entire visual identity of spectrum configurations, luminescence of the samples is provoked by the presence of various defects [32–37].

X-ray luminescence of a powder, obtained from quartz sand, revealed a predominance of internal structural defects associated with an oxygen vacancy (290, 335, 340 nm) over intrinsic defects of a  $\text{SiO}_2$  matrix (400, 460–470 nm), impurity sites of a  $\text{SiO}_4^{3-}/\text{Na}^+$  type, a  $\text{SiO}_4^{3-}/-$  hole site with an exciton-type defect, localized on bond breaking near a structural defect (Ge). Glow bands of a silicon dioxide nano powder, obtained from synthetic quartz glass, are associated with other types of defects. Luminescence is caused by the presence of impurity defects of  $\text{AlO}_4^4/\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{H}^+$  (330–360, 370–390 nm) and, to a lesser extent, with oxygen vacancies (290, 335 nm) and a hole site of  $\text{SiO}_4^{3-}/-$  (420–440 nm). Calcination and X-ray irradiation of samples reduce the glow intensity.

Therefore, general morpho-structural analysis of silica NP from silicon dioxide of different origin revealed differences in both a morphology of the appearance of particles and polymerization of silicon-oxygen chains. In turn, this is an indirect indicator of the presence of distinctive features of the structure [38] and their influence peculiarities. NP globules, obtained from a natural material (quartz sand), are not a sphere with a smooth surface. During polymerization, a three-dimensional nucleus is formed, where part of chains remains on a surface of a spherical particle as active sites. A structural defect, associated with an oxygen vacancy, is noted in this nanopowder from spherical particles, and electric dipoles may be present on a silica surface. This powder has a more harmonious influence on physiological processes proceeding in plants.

NP from a synthetic product (quartz glass – KU-1) form flaky, flat particles that form two-dimensional structures, where Si-O chains are arranged in a configuration that is similar to chain/ layered silicates, on the surface of which there may be an anionic OH-group. The presence of water/OH-groups in their structure is indicated by a band of  $\sim 930\text{--}970\text{ cm}^{-1}$ , which belongs to Si-(OH) valence vibrations, where hydroxyl fluctuates as a single mass and is cationic; that is, hydrogen can be exchanged for any metal cation, which is fixed by luminescence as an impurity defect.

## Conclusions

An assessment of an influence of silicon dioxide nanoparticles of different nature on production of primary and secondary metabolites has shown that monomineral sand nanoparticles more significantly increase the production of both primary and secondary metabolites in plants of the *Poaceae* family, unlike synthetic quartz glass nanoparticles, which influence only the production of primary metabolites.

Exposure to nanoparticles of monomineral sand increases a sum of chlorophylls, flavonoids, hydroxycinnamic acids, carotenoids,  $\alpha$ - and  $\beta$ -chlorophylls, most amino acids in leaves of *Triticum aestivum* (*Poaceae*) and *Avena sativa* (*Poaceae*). Nanoparticles of synthetic quartz glass influence an increase in a content of silicon and some amino acids in leaves and stems of *Triticum aestivum* (*Poaceae*).

An influence of powders of nano particles of different origin on a dynamics of accumulating a sum of flavonoids, hydroxycinnamic acids by the example of the *Fabaceae* family has shown their increase in samples, collected in May.

Morpho-structural analysis of nanoparticles of different origin has revealed a difference in a morphology of particles, polymerization of silicon-oxygen chains, which in turn is an indirect indicator of the presence of distinctive features of a structure and peculiarities of their influence. It has also established that NP globules, obtained from a natural material (quartz sand), are not a sphere with a smooth surface. During polymerization, a three-dimensional nucleus is formed, where part of the chains remains on a surface of a spherical particle as active sites, and NP from a synthetic product (quartz glass – KU-1) form flaky, flat particles, which produce two-dimensional structures.

#### Supplementary Information

The electronic supplement to the article (DOI: <http://www.doi.org/10.14258/jcprm.20260114902s>) provides additional experimental material that reveals the main points set out in the article

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#### Conflict of Interest

The authors of this work declare that they have no conflicts of interest.

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