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Toxicity Assessment of SiO_2 Nanoparticles to Pear Seedlings

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Abstract:
To date, the effects of nanoparticles on woody plants remain unaddressed. This study reveals some of the physiological and biochemical effects of SiO_2 nanoparticles on wild pear seedlings. The seedlings were irrigated with different concentrations of nano silica (0, 10, 100, 500 and 1000 mg/l) for 14 days. Nanoparticle adsorption and absorption, biomass allocation, gas exchange, relative water content, xylem water potential, electrolyte leakage, pigment and proline content, antioxidant enzymes, and nutrient cycles were surveyed. The attachment of nanoparticles on the root surface was observed by scanning electron microscopic and the accumulation of Si in leaves was measured by X-ray fluorescence analysis. Although the performed experiments did not show any acute toxic effects of adding of SiO_2 nanoparticles in irrigation to wild pear plant, the finding should be confirmed with other experiments of longer duration and high exposure concentrations before a final conclusion in this issue can be made.
Keywords*: Nanoparticles, Nano silica, Toxic effect, Woody plants, Physiological-biochemical parameters.*

1. INTRODUCTION

Before using new technologies, their probable effects on all components of natural ecosystems should be surveyed. Nanotechnology is such new technology and it has wide application in chemical, manufacturing, medical, and agricultural sectors [1]. Mass-produced nanoparticles (NP) and nanomaterials (nanometer materials) are a new class of man-made chemical products with unique characteristics. Because of the volume of production, it is likely that nanoparticles will appear in the environment [2]. It should be noted, however, that environmental scientists disagree regarding the phytotoxicity of NP.

Although plants are sessile organisms and cannot escape the effects of NPs in air, water, and soil, most research has focused on microorganisms and animals/ human cells. With regard to higher plants, most of the research has been conducted with seed germination and root elongation bioassays [3, 4, 5, 6]. The tests are limited to annual agricultural and garden crops

[7] with few studies on woody plants [8, 9]. On a global scale, trees account for the majority of biomass [10, 11] and are dominant constituents of terrestrial ecosystems [7]. Trees are characterized by a large woody component, the secondary xylem. The xylem is made up of a continuous porous structure of tracheides or tracheae and xylem elements that are responsible for nutrient and water transport from roots to leaves [10]. The size of these pores in these structures is in the micrometer range. Nanoparticles have a diameter in the range as the name indicates of nanometers and would thus be of a size that may allow xylem transport and accumulation in the xylem structures, eventually blocking the continuity and disturbing or destroying xylem function. This might give woody plants a special vulnerability towards nanomaterials. On the other hand, woody plants have a protective endodermis and the differentially permeable Casparian strip [12], reducing the potential for NP uptake. Currently, many metal oxide nanoparticles and carbon-based materials have phytotoxicity and environmental toxicity [13, 14, 15]. However, the investigations on behavior of nanometal oxides such as nano silica in plants and the mechanism of interaction, its influence, and agricultural application are still in the rudimentary stage [16, 17], and visible Si deficiency or toxicity symptoms are not documented. Thus, plant physiologists have largely ignored it [18]. After oxygen, silicon (Si) is the most abundant element in the earth's crust and using Si instead of herbicides and pesticides could reduce harmful environment effects [19]. In recent years, effects of silicon in nanoscale on herbal plants have received increased attention, but researches on woody plant are still limited.

Considering the importance of trees for global ecosystems, and given the special vulnerability to nanomaterials, it is surprising that no toxicity tests have been done to evaluate the potential hazards of MNP for woody species. On the other hand, plant seedlings are an efficient and useful tool for the bioassay of potentially hazardous chemicals and materials [20]. Consequently, this study was conducted to determine the acute toxic effect of nano silica (SiO_2) on seedlings of pear (*Pyrus biosseriana*).

2. MATERIAL AND METHODS

2.1. Characterization of SiO_2 NPs

SiO_2 nanoparticles were purchased from TECNAN (Tecnología Navarra de Nanoproductos S.L.) company, Spain. The size of NPs was estimated to be 10–15 nm (Figure 1). The XRD measurement clearly showed that used SiO2 NPs were amorphous. The elemental analysis of the nano-powder was performed by ICP-MS technique (THERMO ELEMENTAL VG PQ-ExCell). The purity of SiO_2 NPs calculated with this technique is 99.999%.

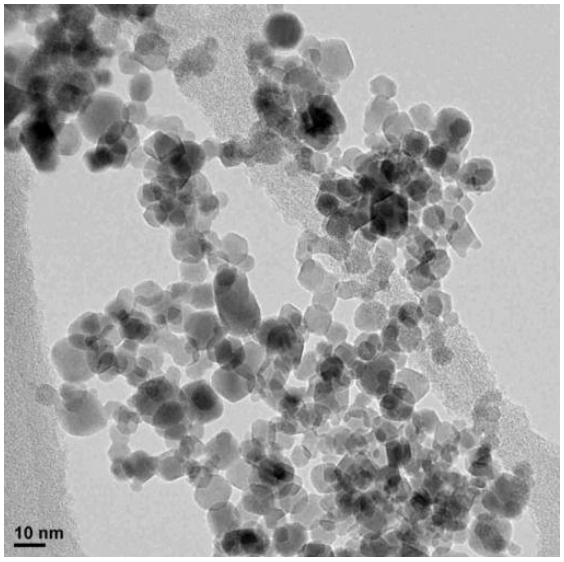


Figure 1. *TEM (JEOL, Mod. JEM-2010) micrographs of nano-SiO_2 powder. The average size of the nanoparticle is 10-15 nm*

2.2. Plant materials and irrigation treatments

The experiment was done at a greenhouse in the faculty of natural resources, Tarbiat Modares University (TMU), Noor, Mazandaran province, Iran. The experimental materials were two-year

old wild pear (P. biosseriana) seedlings. A total of 25 uniformly sized wild pear seedlings were transplanted to plastic pots (5 L) containing a mixture of forest brown soil, river sand, and clay (2:1:1, v/v/v) which were transmitted to the greenhouse. All the seedlings were equally irrigated to field capacity (700 ml per pot) three times per week until the beginning of the experiment, when the plants were treated with a range of nanoparticles concentration. The seedlings were irrigated for 14 days at the NP concentrations of 10, 100, 500, 1000 mg/l and finally 0 mg/l (no NPs as control).

2.3. Measurements of physiological parameters

Net photosynthesis (A, µmol m-2 s-1), stomatal conductance (gs, mmol m-2 s-1), transpiration (E, mmol m-2 s-1) were measured during the experiment period (at days 7, 10, 14) using three randomly selected mature leaves per plant by a portable infrared gas analyzer (Model LCpro+, ADC BioScientific Ltd., Hertfordshire, UK).

Predawn xylem stem potential (ψ stem, MPa) was measured between 04:00 am and 06.00 am at the end of the experiment with a pressure chamber system (Skye, SKPM 1400, UK).

Leaf relative water content (RWC) was determined according to following formula: RWC= (Mf - Md) / (Mt - Md) ×100, where Mf is leaf fresh mass, Mt, turgid mass and Md, dry mass.

To estimate the electrolyte leakage, fresh leaf samples were rinsed 3 times (2-3 min) with distilled water and leaf discs of 0.5 cm^2 were floated in 10mL of distilled water for 24 h and electrical conductivity of the solution was measured by a conductimeter (EC meter- PC 300, Eutech instrument Pte Ltd/ Oakton instruments, USA). Total conductivity was obtained after boiling the samples in a bath (90°C) for 2 h and results were expressed as a percentage of the total conductivity [21] after adjusting for the EC value of the distilled water.

2.4. Assessment of biomass and morphology

The initial value of diameter mean for all plants was 6.52±0.1 mm at the beginning of the experiment. We re-measured the diameter for all plants on the 14th day for assessment of diameter growth. Diameter increase was calculated by subtracting the initial diameter from the final diameter for each seedling. Plants were harvested at the end of the experiment by gently washing the soil from the root system and dividing individual seedlings into roots stems, and leaves.

The root and shoot length were measured with a ruler. At harvest, individual plants were separated into leaves, stems, and roots and oven-dried at 70°C for 72 h, and weighed to obtain their dry weight (DW).

2.5. Measurements of biochemical parameters

On day 14, leaf samples were covered with aluminum foil, frozen in liquid nitrogen and stored at -85°C until they were used for biochemical analysis. Chlorophylls and carotenoids were extracted from leaf samples in 80% acetone and their contents were determined by spectrophotometry according to Gholami *et al*. [22].

Free proline content in leaves was quantified according to original methodology of Bates *et al*. method [23]. The protocol is based on the formation of red colored formazone by the reaction of proline and ninhydrin in acidic organic solvent like toluene.

A peroxidase and catalase enzyme essay was done according to a procedure proposed by Ebermann and Stich [24].

2.6. Measurements of leaf nutrient elements

Oven-dried leaves were pulverized in an electric mill. The powdered leaves were transmitted to

the atomic energy organization of Iran (AEOI). The concentrations of SiO_2 NPs and the other elements were detected by X-ray fluorescence (XRF) (ED 2000 Oxford Instruments Corporation) analysis.

2.7. Microscopical investigations

At the end of the experiment, the fresh root sections were taken for microscopic analysis. The adsorption of SiO_2 NPs to fresh roots was observed by scanning electron microscopy (SEM) (KYKY-EM3200) in the laboratory of Tarbiat Modares University.

2.8. Statistical analysis

Morphological, physiological and biochemical data were analyzed using a fixed effects model and one-way ANOVA. Variations in leaf gas exchange parameters during the experiment were evaluated by repeated measures ANOVA. All statistical analyses were performed with SPSS 16.0 (SPSS Inc., Chicago, IL).

3. RESULTS

3.1. Growth and morphological parameters

The effect of different concentrations of SiO_2 NPs on growth and biomass of pear seedlings was evaluated and shown in Figure 2. A significant increase or decrease in the biomass allocation of pear plants was not observed in different concentrations of SiO_2 NPs compared with the control. The average root length of the plants was not significantly different among the SiO_2 NPs treatment; also, diameter growth of treated plants was not affected by SiO_2 NPs concentrations.

3.2. Physiological parameters

Repeated measures ANOVA showed that net photosynthesis rate (A) and stomatal conductance (gs) were not affected by SiO_2 nanoparticle levels and date of experiment. On the one hand, increased $NSiO_2$ concentrations

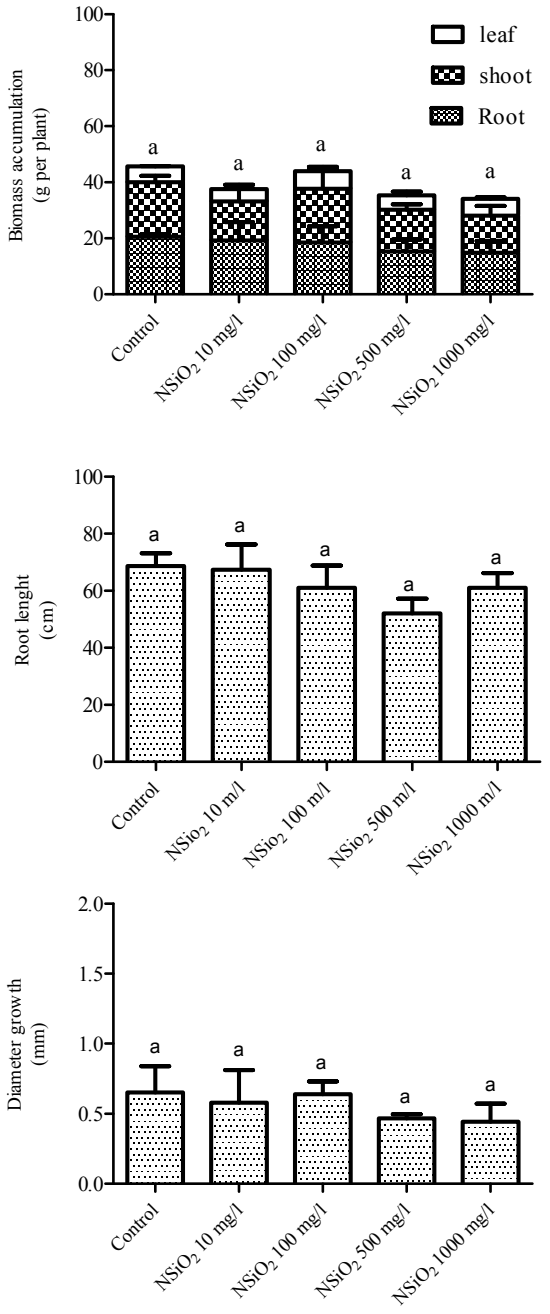


Figure 2. *Effect of irrigation with different concentrations of nano silica on growth parameters after 14 days of growth. Same letters indicate no significant differences (P≥ 0.05) among treatments based on the Duncan test. (Mean ± SE; n=5)*

during watering reduced the transpiration rate (E) (Figure 3). When NSiO2 was applied to the irrigation water, the xylem water potential changed significantly (Figure 4). Xylem water potential of wild pear seedlings decreased with increasing levels of nano particles. Increased levels of $NSiO_2$ corresponded with significantly decreased RWC. Leaf membrane stability increased slightly with addition of nano particles, while the highest values were observed with high levels of NSiO2 (Figure 4).

3.3. Biochemical parameters

SiO_2 NPs did not affect the leaf pigment contents (Table 1). It can be seen that SiO_2 NPs did not change the proline content of the leaf samples (Table 1). Enzyme activity of pear plants was strongly affected by SiO_2 NPs treatments (Table 1). Increased SiO_2 NPs levels caused an increase in peroxidase and catalase activity; the strongest increase was observed mainly at the higher levels of SiO_2 NPs, compared with the control. At 1000 mg/l SiO_2, peroxidase activity was greater in treated plants than in control plants. 500 mg/l N-Si increased the catalase activity up to 65% (Table 1).

3.4. Leaf nutrient elements

Increased SiO_2 NPs in irrigation water had variable effects on the concentration of nutrients (Figure 5). As might be expected, the highest Si content was recorded in tissues of SiO_2 NPs irrigated plants, but there was no statistical difference between SiO_2 NPs treatments. Generally, application of SiO_2 NPs in watering increases the Si uptake by 40-60% in the roots. The nitrogen uptake by roots of pear plants decreased with the addition of SiO_2 NPs. Similarly, the phosphorus concentration declined with addition of SiO_2 NPs. On the other hand, the highest K content was recorded at the concentration of 1000 mg/l $NSiO_2$, so it seems that uptake of K increased with adding SiO_2

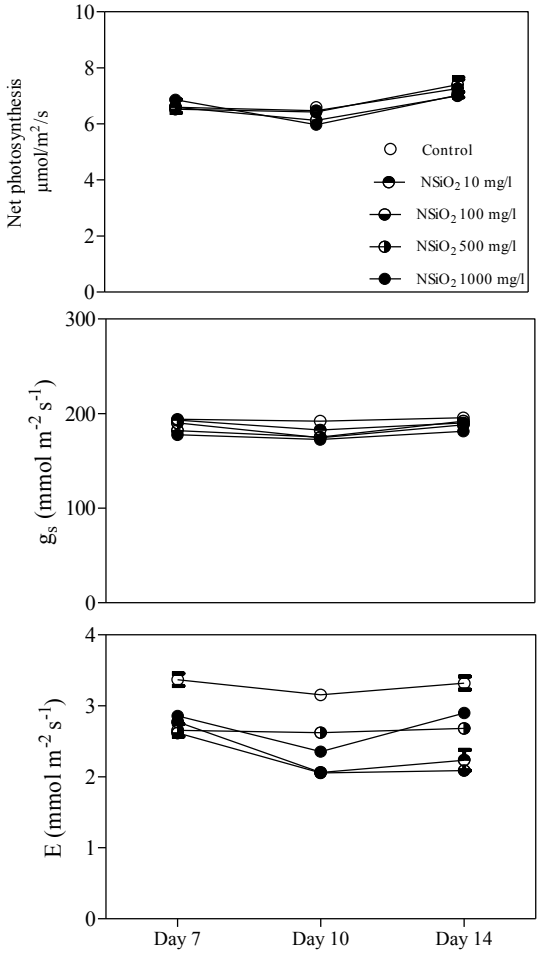


Figure 3. *Effect of irrigation with different concentrations of nano silica on gas exchange parameters. The gas exchange was assayed with a portable infrared gas analyzer, at days 7, 10 and 14 on the recent fully expanded leaves of all plants in similar positions on the plant, (Mean ± SE; n=5).*

NPs. Although Ca uptake increased by the application of SiO_2 NPs, the uptake of Fe and Al declined.

3.5. Microscopic analysis

Figure 6 shows photographs of pear roots and SiO_2 NPs aggregates on the root surface. The roots were exposed to different concentrations of

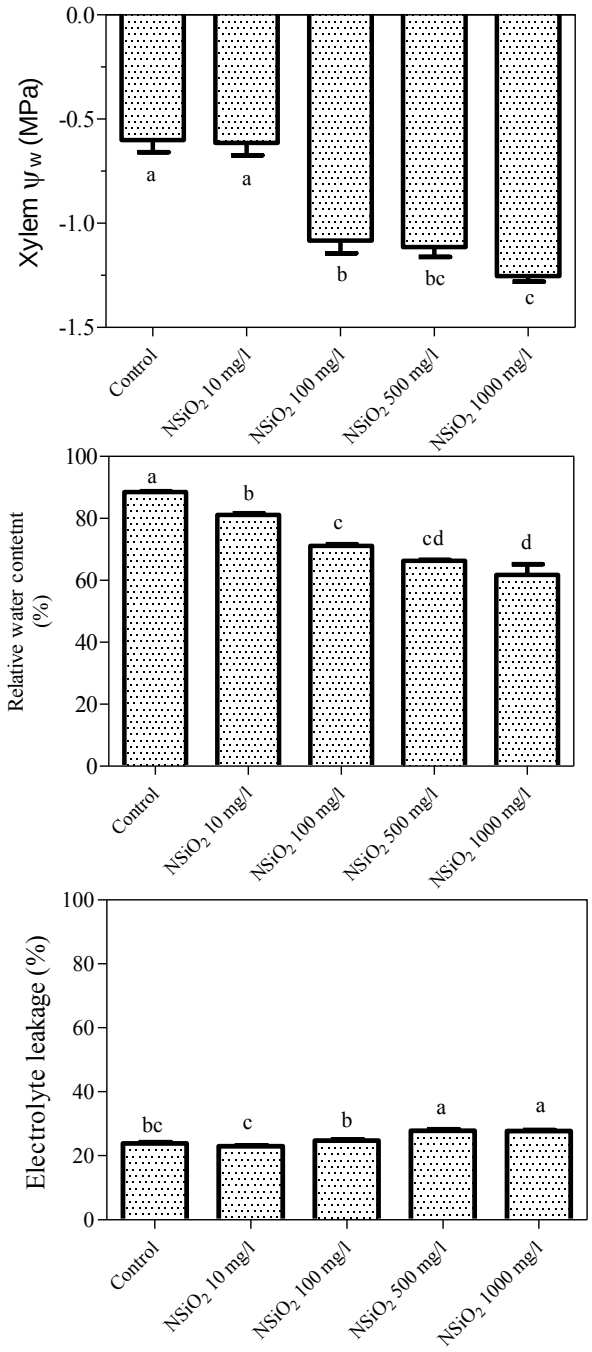


Figure 4. *Effect of irrigation with different concentrations of nano silica on some physiological parameters after 14 days of growth. Different letters indicate significant differences (P< 0.05) among treatments based on the Duncan test. (Mean ± SE; n=5)*

SiO_2 NPs in the 10-1000 mg/l concentration range. In particular, for the higher concentrations (500 and 1000 mg/l), nanoparticles attached to the roots could be observed, whereas only a very small amount of particles were found to be attached to the roots for $NSiO_2$ at 10 and 100 mg/l. There was no sign of nanoparticles on roots of control plants. Clearly, the particles on the root surface are in the nanometer range (for comparison: a bar is 1 μm in length).

4. DISCUSSION

The use of nano-compound materials has been given much attention by plant biological researchers [25, 26]. It should be noted that the data on phytotoxicity of NP are sometimes contradictory. In some studies, no visible signs of SiO_2 NPs phytotoxicity were detected; in other studies, the negative effects of the nanoparticle on plants were reported. For example, Lu *et al*. [27] studied the effects of a mixture of SiO_2 and TiO_2 nanoparticles on soybean (*Glycine max*) and reported no toxic effect on the species. Lin *et al*. [28] tested TMS (nanostructured silicon dioxide) on growth of Changbai larch (*Larix olgensis*) seedlings. They observed that *Larix* seedling growth and quality increased with TMS. Plants treated with 500 $\mu L.L^{-1}$ TMS showed the greatest mean height, root collar diameter, main root length, and number of lateral roots of seedlings. Although there was a report of toxicity of N-SiO_2 on *Arabidopsis thaliana*, this toxicity was not as strong as that of other nanoparticles, such as N-ZnO and N-Fe_3O_4 [29]. Haghighi and Pessarakli [25] observed a decrease of fresh and dry weight and root volume of tomato at high levels of N-Si when compared with control plants. Their results showed that N-Si did not significantly affect stem diameter, water uptake, or RWC. Similar results were found in Arabica coffee plants, where high soil concentrations of calcium silicate reduced root growth [30].

Table 1. *Effect of irrigation with different concentrations of nanosilica on some biochemical parameters after 14 days of growth. Different letters indicate significant differences (P< 0.05) among treatments based on the Duncan test. (Mean ± SE; n=3)*

Biochemical parameters	Control	SiO_2 10 mg/l	SiO_2 100 mg/l	SiO_2 500 mg/l	SiO_2 1000 mg/l
Chlorophyll a (µg/FW)	0.005±0.000	0.004±0.000	0.003±0.001	0.005±0.000	0.005±0.000
Chlorophyll b (µg/FW)	0.006±0.001	0.004±0.000	0.004±0.000	0.005±0.000	0.005±0.000
Chlorophyll a+b (µg/FW)	0.004±0.000	0.004±0.000	0.003±0.000	0.004±0.000	0.004±0.000
Carteon (µg/FW)	0.647±0.092	0.484±0.018	0.582±0.094	0.588±0.060	0.502±0.072
Proline (µg/FW)	76.72±0.963	61.33±2.581	66.59±7.663	59.44±2.764	64.55±1.791
Peroxidase	0.0001±0.000c	0.0007±0.000 a	0.0002±0.000bc	0.0003±0.000b	0.0008±0.000 a
Catalase	0.897±0.034 c	0.881±0.026 c	2.610±0.105 a	2.618±0.067 a	1.868±0.206 b

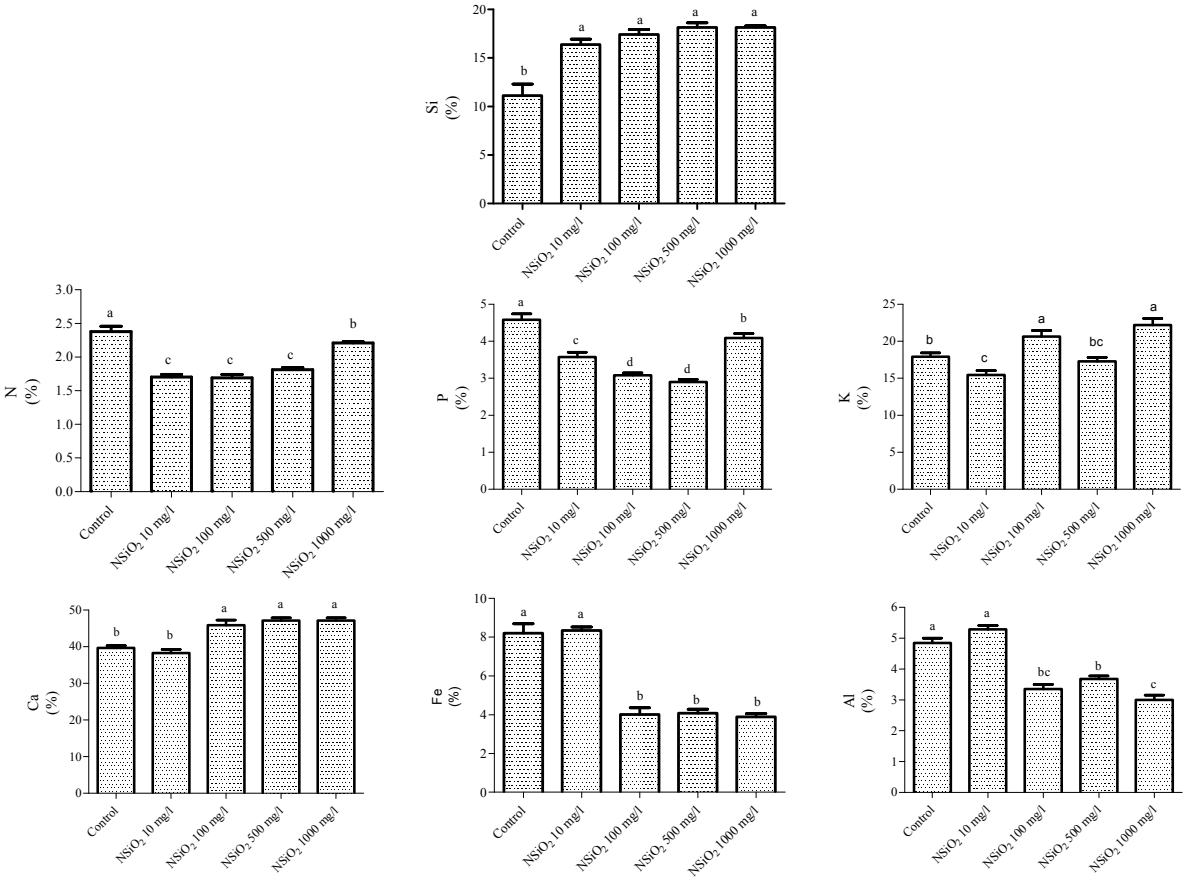
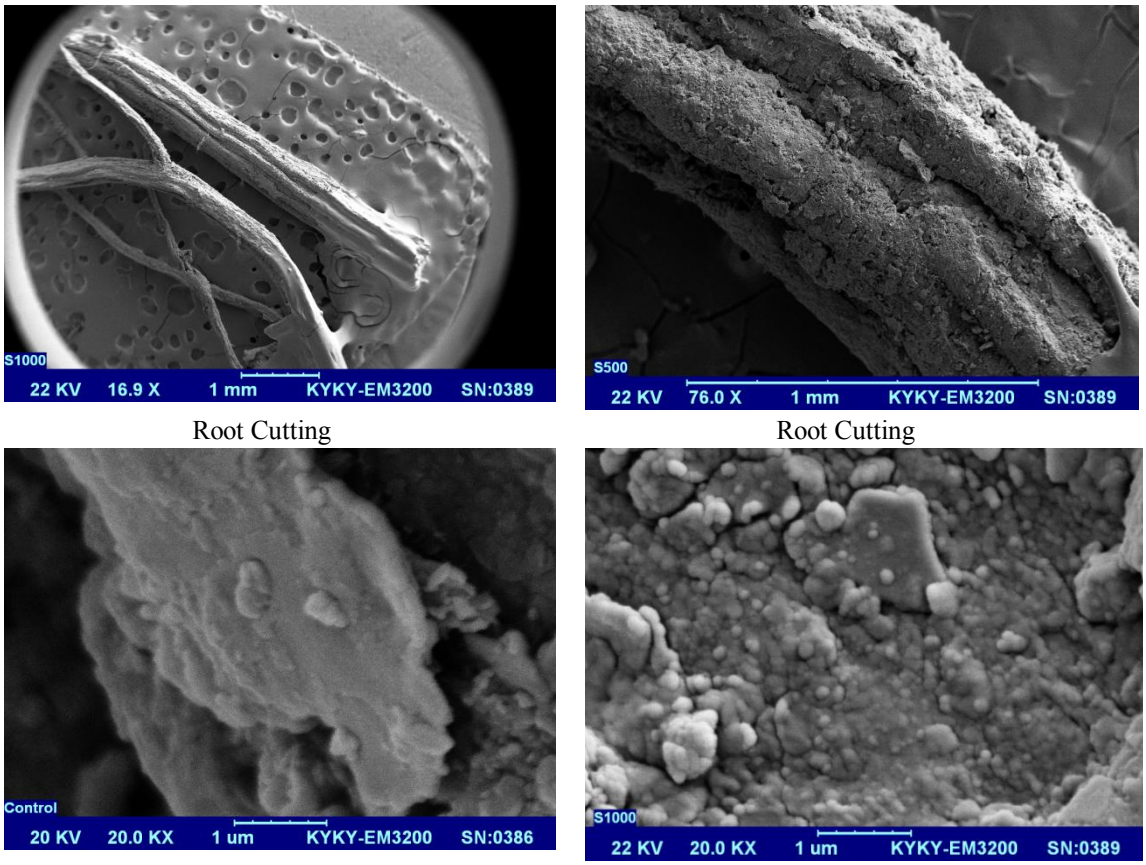


Figure 5. *Effect of irrigation with different concentrations of nano silica on nutrient elements after 14 days of growth. Different letters indicate significant differences (P< 0.05) among treatments based on the Duncan test. (Mean ± SE; n=3)*



Root Cutting Root Cutting

Control $NSiO_2$ 1000 mg/l

Figure 6. *Detection of nanoparticles on root surface by scanning electron microscopy (SEM). The absence of nanoparticles on root epidermis in control seedlings (left). The presence of nanoparticles on root epidermis in 1000 mg.L-1 seedling SiO_2 treatment (right).*

In the current research, we examined the probable toxic effects of SiO_2 NPs at three aspects of biomass allocation, physiological and biochemical. We observed insignificant and negligible negative effect of SiO_2 NPs on growth and biomass allocation of the seedlings. This result was also seen in root length and diameter growth. It should be noted that SiO_2 NP concentrations did not have any effect on morphological and growth characters, possibly because of short-term time treatments of SiO_2 NPs.

Although application of SiO_2 NPs in watering could not negatively affect photosynthesis rate and stomatal conductance, Transpiration rate declined compared to control plants. Silica precipitates in tissues with photosynthesis activity [31, 32] as a boundary layer water loss via stomata and finally leads to decreasing of transpiration [33]. Silicon is an element that does not cause severe injury to plants when present in excess and can provide multiple benefits [34]. It seems decreasing the transpiration rate is one of the beneficial effects of silicon on plants.

Ma and Yamaji [31] believed that xylem loading plays an important role in accumulation of Si in rice shoots. The slight decrease of xylem water potential with the application of high levels of nano silica may be attributed to xylem loading. Decreased relative water content (RWC) in SiO_2 NPs treatments was correlated with a decrease of xylem water.

The mechanism of toxicity of NPs is unknown, but generation of ROS and oxidative stress are signs of toxicity [35]. The release of 50% of total electrolytes from plant tissue is considered an index of cell death [36]. We

observed a very slight increase in electrolyte leakage after irrigation of seedlings with high levels of SiO_2 NPs. Thus, it can be noted that SiO_2 NP concentrations did not induce oxidative damage and cell death in the leaves of wild pear seedlings. In addition, the exogenous silicon significantly enhances the activity of superoxide dismutase, peroxidase, and catalase presence in barley roots [37]. Similarly, we clearly observed an increase of antioxidant enzymes in leaves of wild pear when subjected to high levels of nano SiO_2. On the other hand, the proline content was not affected by the treatments. Generally, the role of nano silica in plant biology has been poorly understood and the attempts to associate Si with metabolic or physiological activities have been inconclusive [38].

The change of chlorophyll contents happened much earlier than growth. Therefore, chlorophyll, especially chlorophyll a, could be served as a more sensitive indicator than growth inhibition and be used for early warning of NPs exposure [39]. It can be concluded that there was not a negative effect on plant architecture with unchanged contents of chlorophyll due to application of $NSiO_2$ in irrigation. Silicon (Si) accumulation differs greatly among plant species because of differences in Si uptake by the roots [40, 18]. However, SiO_2 nanoparticles were adhered in large numbers to the root surface of wild pear seedlings, especially at high concentrations; translocation of Si from root to leaf remained at the same level under different concentrations. Plants differ greatly in their ability to accumulate Si, ranging from 0.1% to 10.0% of Si (dry weight) [41, 42]. Generally, the Si concentration increased about 47-63% in leaves of wild pear seedlings when subjected to SiO_2 NP compared with control seedlings. Si NPs can raise the pH of growth medium such as soil [43]. On the other hand, a more basic pH may limit the availability and absorption of nutrients [44]. Alleviation of K and Ca contents has occurred due to increased SiO_2 NPs concentration, while N, P, Fe and Al contents have declined due to applied SiO_2 NPs in irrigation. The role of silica in ameliorating P and K contents has been explained in earlier reports [45, 46].

5. CONCLUSION

In summary, some negative effects have been observed after exposure of plants to SiO_2 nanoparticles, while the observed effects were quite small in our study. The wild pear seedlings were not sensitive to short-term exposure to SiO_2 NPs. Similar results were reported by Seeger *et al*. [7] for willow cuttings in response to TiO_2 nanoparticles. Although the performed experiments did not show any acute toxic effects of adding SiO_2 in irrigation to wild pear plant, the finding should be confirmed by other experiments of longer duration and high exposure concentrations before a final conclusion in this issue can be made.

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