

# Morphological, Physiological and Biochemical Responses of Crops (*Zea mays* L., *Phaseolus vulgaris* L.), Medicinal Plants (*Hyssopus officinalis* L., *Nigella sativa* L.), and Weeds (*Amaranthus retroflexus* L., *Taraxacum officinale* F. H. Wigg) Exposed to SiO<sub>2</sub> Nanoparticles

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## ABSTRACT

In this research, two field crops (*Zea mays* L. and *Phaseolus vulgaris* L.), two medicinal plants (*Hyssopus officinalis* L. and *Nigella sativa* L.) and two weeds (*Amaranthus retroflexus* L. and *Taraxacum officinale* F. H. Wigg) were separately treated with three concentrations of SiO<sub>2</sub> nanoparticles (400, 2,000, and 4,000 mg L<sup>-1</sup>). The effects of these treatments on morphological and biochemical characteristics of the plants were assessed, including germination, root and shoot length, root and shoot fresh weight, root and shoot dry weight, photosynthetic pigments, total carbohydrates, total protein, total amino acid, and proline content. In the crops and medicinal plants, 400 mg L<sup>-1</sup> SiO<sub>2</sub> NPs significantly increased seed germination, root and shoot lengths, fresh weights (except for *H. officinalis*) and dry weights, photosynthetic pigments, total protein, and total amino acid (except for *H. officinalis*). In weeds, as SiO<sub>2</sub> NP concentration increased from 400 to 4,000 mg L<sup>-1</sup>, germination, root and shoot lengths, fresh and dry weights, and photosynthetic pigments as well as total protein decreased. Total carbohydrates in all plants decreased significantly, except for *A. retroflexus* at 400 mg L<sup>-1</sup> SiO<sub>2</sub> NPs. In all plant species, with increasing SiO<sub>2</sub> NP concentration, proline content increased significantly. According to these results, a lower concentration of SiO<sub>2</sub> NPs can have beneficial effects on morphological, physiological, and biochemical characteristics of plants.

**Keywords:** Germination, Photosynthetic pigments, Total amino acid, Total carbohydrates, Total protein.

## INTRODUCTION

Nanobiotechnology is one of most important developing sciences. The uses of products from this technology have increased in agriculture, industry, medicine and the military (Gruère, 2012; Qu *et al.*, 2013; Sharifi-Rad *et al.*, 2014). Nanotechnology has many uses in all steps of processing, production, packaging, storing, and transport of agricultural products. The use of

nanotechnology in agriculture also has environmental benefits and, as an interdisciplinary science, can be used as a powerful tool to empower the agricultural sector and in important cases such as crop production, use less pesticides and fertilizers to maintain crops for longer periods (Chinnamuthu and Boopathi, 2009; Mousavi and Rezaei, 2011).

With the rapid development of nanotechnology and its applications, nano-structured materials have been widely used in the fields of

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biomedicine, pharmaceutical, and other industries (Maghabl *et al.*, 2012; Cao *et al.*, 2013; Rad *et al.*, 2013a; Rad *et al.*, 2013b). Nanometer silicon dioxide (nano-SiO<sub>2</sub>) is one of the most popular nanomaterials used in industrial manufacturing, packaging, synthesis of high-molecule composite materials and ceramics, disease labeling, drug delivery, cancer therapy and biosensors (Sahoo *et al.*, 2007). Most experiments to date that have assessed the effects of SiO<sub>2</sub> NPs on plants have considered physiological, biochemical or morphological characteristics of usually one or maximum two plants (Slomberg and Schoenfisch, 2012; Siddiqui and Al-Whaibi, 2014). Generally, studies on the effects of Si NPs on plants are limited and study on how the same levels of exposure to SiO<sub>2</sub> NPs would affect a wide range of crops using morphological, physiological and biochemical characteristics is lacking.

There are some important crops that can be included in such a study. For example, maize (*Zea mays* L.; Poaceae), one of the most widely cultivated crops, is a major component in the diet of many developing countries such as Iran and it is one of the crops with the most biotechnological potential for energy production and other industrial applications (McLaren, 2005). Also, common bean (*Phaseolus vulgaris* L.; Fabaceae), which is the most important grain legume and plays an important role in human nutrition as a valuable source of minerals, protein, fibres, calories and vitamins, is produced in a wide range of climatic conditions in Iran and elsewhere (Sadeghi and Cheghamirza, 2012). Medicinal plants can also be included in such a study: *Hyssopus officinalis* L. (hyssop; Lamiaceae), a perennial plant with a long history of traditional and medicinal uses, is an endemic Iranian species of the genus *Hyssopus* (Khazaie *et al.*, 2008). Also, traditionally, *H. officinalis* – named Zufa in Iran – has been used as a tonic, antiseptic, cough reliever carminative and expectorant (Khazaie *et al.*, 2008). In spite of having a somewhat bitter taste, *H. officinalis* is frequently used as a condiment and minty flavor in the food industry. Black seed (*Nigella sativa* L.; Ranunculaceae) has a long history of traditional medicinal use. It is also used as a flavouring agent and food additive in many countries, especially in developing countries (Sharifi-Rad *et al.*, 2014). Black seed oil is

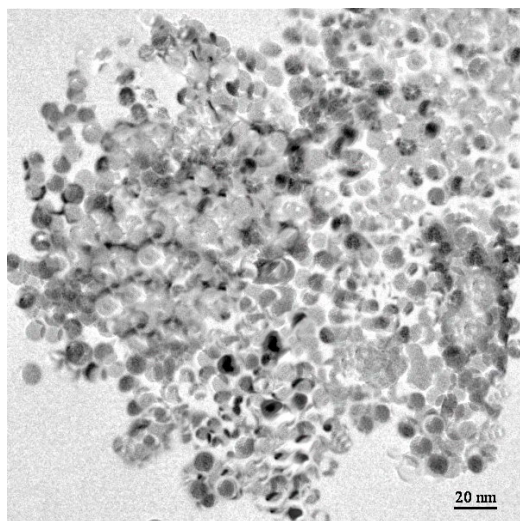
reportedly beneficial due to its content of over 100 components such as vitamins, trace elements, and aromatic oils (Ali and Blunden, 2003). To further diversify the kind of crops for this type of research, weed plants can be studied. For example, redroot amaranth (*Amaranthus retroflexus* L.; Amaranthaceae) is a critically destructive weed distributed worldwide, particularly in farmlands, wastelands and gardens that is difficult to control due to its extreme vigor, flexibility and prolific seed production. Redroot amaranth can seriously influence the growth of crops and pollute crop seeds, causing immense losses to agricultural production (Costea *et al.*, 2004). Also, dandelion (*Taraxacum officinale* F. H. Wigg; Asteraceae), a weed and perennial herbaceous plant that is native to the entire northern hemisphere, has several varieties and subspecies and grows as weed in wild, moist pastures in temperate areas (Chaitanya *et al.*, 2013).

This study, the first of its kind for abovementioned set of plants, aimed to assess how the same levels of exposure to SiO<sub>2</sub> NPs would affect a wide range of crops using morphological, physiological and biochemical characteristics. The plants considered for the present study were two field crops (*Zea mays* L., *Phaseolus vulgaris* L.), two medicinal plants (*Hyssopus officinalis* L., *Nigella sativa* L.), and two weeds (*Amaranthus retroflexus* L., *Taraxacum officinale* F. H. Wigg) so as to have a wider and more representative assessment of the impact of SiO<sub>2</sub> NPs on plants.

## MATERIALS AND METHODS

### Plant Materials and SiO<sub>2</sub> NP Treatments

Seeds of maize (var. 'KSC 704'), common bean (cv. 'Naz'), hyssop (var. *angustifolius*), black cumin (cv. 'Baft'), redroot pigweed (var. *retroflexus*) and dandelion were obtained from the Iranian Agricultural Organization, Zabol City, Iran. SiO<sub>2</sub> NPs were purchased from Sigma-Aldrich (St. Louis, MO, USA; 99.5% purity; 10-20 nm in size; white; spherical shape; density of 3.8 g cm<sup>-3</sup>). Figure 1 shows a Transmission Electron Microscopic (TEM) image of the tested NPs. The NPs were dispersed



**Figure 1.** Transmission Electron Microscopy (TEM) image of SiO<sub>2</sub> NPs used in this study (Sigma-Aldrich).

in distilled water at three concentrations (400, 2,000 and 4,000 mg L<sup>-1</sup>) and then sterilized at 120°C for 20 minutes. An A KQ 5200DE model ultrasonicator (Shumei Instrument Factory, Kunshan, China), applied at 60 Hz for 30 minutes, was used for easy dispersion of NPs without precipitation. For the best dispersion, SiO<sub>2</sub> NPs were sufficiently shaken after sonication to break up agglomerates. For each SiO<sub>2</sub> NP treatment (400, 2,000 and 4,000 mg L<sup>-1</sup>), each concentration was prepared separately, without dilution, by weighing NPs and dispersing them in distilled water. The SiO<sub>2</sub> NP suspensions were dispersed by sonication for 20 min before use. Seeds were surface sterilized by incubating for 20 minutes in 5% (w/v) sodium hypochlorite followed by four washes with sterile distilled water. Seeds were treated with 5 mL at three concentrations (400, 2,000 and 4,000 mg L<sup>-1</sup>) of SiO<sub>2</sub> NPs on a double layer of wet filter paper in Petri dishes at 20 seeds/plant/Petri dish. Distilled water was used as the control. Seeds were germinated at 25±1°C and placed in the dark. After 24 hours, germination was checked and recorded for each treatment. Fourteen days after the initiation of seed germination under these conditions, the length (mm) of shoots and roots was measured. Healthy and uniform (in size and appearance) 14-day-old seedlings were transferred to pots (30×25 cm). The pots were filled with fertile loam soil (~0.5

kg; pH= 5.6; Electrical conductivity = 12 meq 100 g<sup>-1</sup>) up to three-quarters of the height of the pot. Each test pot was supplied daily with 10 mL of freshly prepared SiO<sub>2</sub> NPs at each concentration for 16 days (i.e., until 30 days of age) along with the control. The control was distilled water without SiO<sub>2</sub> NPs. Plants were grown in strictly controlled conditions (25±1°C, 16-hour photoperiod, 350 μmol m<sup>-2</sup> s<sup>-1</sup> photosynthetic photon flux density and 70% relative humidity) in a greenhouse. Crops (*Z. mays*, *P. vulgaris*) were planted at a density of 10 pot<sup>-1</sup>, medicinal plants (*H. officinalis*, *N. sativa*) at 25 pot<sup>-1</sup>, and weeds (*A. retroflexus*, *T. officinale*) at 30 pot<sup>-1</sup>. Sixteen days after the transfer of seedlings into pots, the root and shoot Fresh Weight (FW), root and shoot Dry Weight (DW), photosynthetic pigments, total carbohydrates, total protein, total amino acids, and proline content were measured, as detailed next.

### Root and Shoot Length

After 14 days, root and shoot length were measured in mm with a ruler.

### Fresh and Dry Weight of Seedling Shoots and Roots

The shoot and root FW of 30-day-old seedlings was assessed for all treatments since, by 30 days, plants had well developed leaf and root systems suitable for transplanting. The DW of shoots and roots was determined after placing the entire plantlets (with organs divided) in an oven for 48 hours at 75°C. Shoot and root DW were expressed as g pot<sup>-1</sup>, measured with 10, 25, and 30 seedlings pot<sup>-1</sup> for crops, medicinal plants and weeds, respectively, and four replicates per treatment.

### Measurement of Photosynthetic Pigments

The content of photosynthetic pigments in control and treated plants was measured according to Lichtenthaler *et al.* (1987). Two hundred mg of randomly selected leaf tissue



was weighed and pulverized with a mortar and pestle in liquid nitrogen. Large pieces were completely pulverized with 80% acetone, and the final volume was brought to 25 mL. The resulting solution was centrifuged in a refrigerated Beckman GS-15R centrifuge (365702; bench-model; Ontario, Canada) at 4,800 rpm for 20 minutes. The supernatant was used to measure the content of chlorophyll (chl) *a*, *b* and carotenoids. Light absorption of the plant extracts was determined by a Shimadzu A160 spectrofluorometer (Shimadzu, Japan) at 470, 645, 646.8, 663 and 663.2 nm.

### Total Carbohydrate Determination

In this study, carbohydrate content was determined by the phenol-sulfuric acid method (Rao and Pattabiraman, 1989). Briefly, to 1 mL of each leaf aqueous extract, 50  $\mu$ L of 80% phenol (Merck, Darmstadt, Germany) and then 3 mL of 98% sulfuric acid (Merck) were added. The leaf aqueous extract was prepared by crushing 2 g of fresh leaf tissue with 3 mL of distilled water then centrifuging the resulting solution for 10 minutes at 200 rpm, separating the supernatant and using it for the next steps of the experiment. The mixture was vortexed for 1 min, then, kept at room temperature for 30 minutes. The absorbance was read at 490 nm with the same spectrophotometer.

### Total Protein Determination

In this study, total protein was extracted from the leaf tissue of each plant by homogenization with a mortar and pestle on ice at 4°C in an extraction buffer containing 50 mM Tris-HCl (pH 7.5), 2 mM EDTA, 0.5 mM EGTA, 1 mM PMSF and 1% Triton X-100. The homogenates were centrifuged in a Beckman GS-15R centrifuge at 14,000 rpm for 10 minutes at 4°C. The soluble protein concentration in the homogenate supernatant was determined using Bovine Serum Albumin (BSA) (Merck) as standard (Bradford, 1976).

### Total Amino Acid Assay

Total amino acids were determined by reaction with ninhydrin using glycine as standard according to Sun *et al.* (2006). Briefly, to 500  $\mu$ L solution of amino acids, 1 mL of 80% acetic acid and 1 mL of ninhydrin solution (2 mg ninhydrin in 50% ethanol) were added, then, mixed for 15 minutes. This mixture was placed at 100°C for 15 minutes and then at 70°C for 10 minutes. Finally, 5 mL of 2-propanol (50%) was added and absorbance was read at 570 nm. Different concentrations of the amino acid glycine were used to create a standard curve.

### Free Proline Amino Acid Content Assay

Proline was extracted and measured according to the method of Bates *et al.* (1973). Leaf samples (0.8 g) were extracted with 3% sulphosalicylic acid. One mL of extracts was placed for 1 hour in boiling water. Then, 2 mL ninhydrin, 2 mL glacial acetic acid, and 4 mL of ice-cold toluene were added sequentially. Proline content was measured by a Shimadzu UV 1601 spectrophotometer at 520 nm and calculated as  $\mu$ mol g<sup>-1</sup> DW against a proline standard (Sigma-Aldrich).

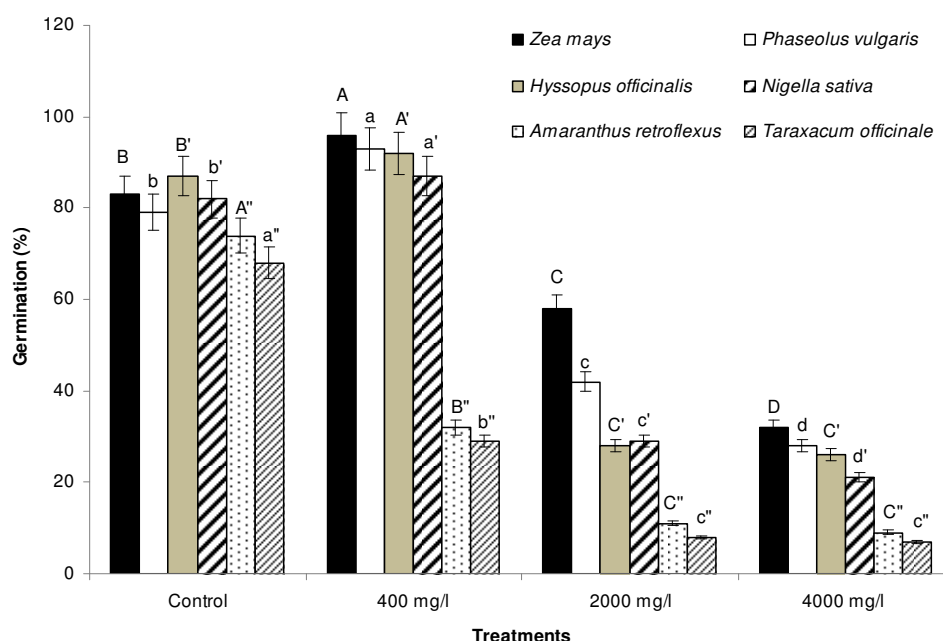
### Statistical Analysis

All data were analyzed as a completely randomized design with four replications. Data were expressed as means  $\pm$  Standard Error (SE). Means between treatments were separated by Analysis Of Variance (ANOVA) and then statistically significant differences between means were assessed by Duncan's new Multiple Range Test (DMRT) and SPSS software version 11.5 (IBM SPSS, New York, USA) at  $P \leq 0.05$ .

## RESULTS

### Effects of SiO<sub>2</sub> NPs on Seed Germination

The effects of SiO<sub>2</sub> NPs on seed germination are shown in Figure 2. In all six plants, there



**Figure 2.** Effect of nano-SiO<sub>2</sub> nanoparticles (400, 2,000, and 4,000 mg L<sup>-1</sup>) and the control (distilled water without nano-SiO<sub>2</sub>) on seed germination in six test species. Different lower case and capital letters show significant differences (DMRT) between treatments means for any one plant species at  $P \leq 0.05$ .  $n = 4$ .

were significant differences between the control and all treatments: 400 mg L<sup>-1</sup> SiO<sub>2</sub> NPs significantly stimulated seed germination more than the control, but 2,000 and 4,000 mg L<sup>-1</sup> SiO<sub>2</sub> NPs significantly reduced seed germination relative to the control and 400 mg L<sup>-1</sup> SiO<sub>2</sub> NPs.

### Effects on Root and Shoot Length

The effects of SiO<sub>2</sub> NPs on root and shoot length are shown in Table 1. In *Z. mays*, *P. vulgaris*, *H. officinalis* and *N. sativa*, there was a significant difference between the control and all treatments with 400 mg L<sup>-1</sup> SiO<sub>2</sub> NPs, which significantly stimulated root and shoot length more than the control. However, 2,000 and 4,000 mg L<sup>-1</sup> SiO<sub>2</sub> NPs significantly reduced root and shoot length relative to the control and 400 mg L<sup>-1</sup> SiO<sub>2</sub> NPs. In *A. retroflexus* and *T. officinale*, the control resulted in significantly longer roots and shoots than when any concentration of SiO<sub>2</sub> NPs was used, except for 400 mg L<sup>-1</sup> SiO<sub>2</sub> NPs in *A. retroflexus*.

### Effects on Root and Shoot Fresh and Dry Weight

The effects of SiO<sub>2</sub> NP concentration on root and shoot FW and DW are shown in Table 2. In both crops and in both medicinal plants, in most cases, there was a significant difference in root FW and DW between the control and all treatments: 400 mg L<sup>-1</sup> SiO<sub>2</sub> NPs significantly stimulated root and shoot FW and DW more than the control, but 2,000 and 4,000 mg L<sup>-1</sup> SiO<sub>2</sub> NPs significantly reduced root and shoot FW and DW relative to the control and 400 mg L<sup>-1</sup> SiO<sub>2</sub> NPs. Notable differences were for *H. officinalis* shoot FW, where the control and 400 mg L<sup>-1</sup> SiO<sub>2</sub> NPs were not significantly different, and for *H. officinalis* root DW, and *N. sativa* root FW and DW, in which 2,000 and 4,000 mg L<sup>-1</sup> SiO<sub>2</sub> NPs were not significantly different. The two weeds showed a different trend to the crops and medicinal plants, with the control resulting in significantly higher root and shoot FW and DW than all SiO<sub>2</sub> NP concentrations, except for A.



**Table 1.** Effect of SiO<sub>2</sub>NPs on Root Length (RL) and Shoot Length (SL) (in mm) of six test species,<sup>a</sup>

SiO <sub>2</sub> (mg L <sup>-1</sup> )	<i>Zea mays</i>		<i>Phaseolus vulgaris</i>		<i>Hyssopus officinalis</i>		<i>Nigella sativa</i>		<i>Anaranthus retroflexus</i>		<i>Taraxacum officinale</i>	
	RL (mm)	SL (mm)	RL (mm)	SL (mm)	RL (mm)	SL (mm)	RL (mm)	SL (mm)	RL (mm)	SL (mm)	RL (mm)	SL (mm)
Control (0)	12.6 ± 0.09 b	29.3 ± 0.04 b	11.6 ± 0.12 b	19.4 ± 0.14 b	8.6 ± 0.09 b	12.4 ± 0.09 b	9.6 ± 0.09 b	13.2 ± 0.16 b	3.8 ± 0.12 a	9.4 ± 0.14 a	2.9 ± 0.14 a	6.7 ± 0.14 a
400	17.7 ± 0.14 a	37.4 ± 0.12 a	17.3 ± 0.09 a	25.6 ± 0.09 a	13.2 ± 0.12 a	18.2 ± 0.12 a	14.3 ± 0.14 a	19.2 ± 0.10 a	3.7 ± 0.09 a	6.2 ± 0.09 b	1.9 ± 0.07 b	4.2 ± 0.16 b
2000	9.4 ± 0.08 c	18.3 ± 0.09 c	7.4 ± 0.07 c	14.3 ± 0.08 c	4.1 ± 0.14 c	10.3 ± 0.09 c	5.1 ± 0.18 c	8.3 ± 0.12 c	2.2 ± 0.12 b	3.8 ± 0.09 c	1.1 ± 0.12 c	2.1 ± 0.07 c
4000	5.6 ± 0.1 d	14.6 ± 0.07 d	4.2 ± 0.10 d	11.2 ± 0.14 d	3.9 ± 0.07 c	9.8 ± 0.09 d	3.4 ± 0.08 d	6.2 ± 0.14 d	1.1 ± 0.09 c	2.2 ± 0.08 d	0.9 ± 0.09 c	2.0 ± 0.14 c

<sup>a</sup> Values indicate mean±SE. Different lower case letters indicate significant differences (DMRT) in RL and SL (assessed separately) for each plant species across treatments ( $P \leq 0.05$ ),  $n = 4$ .

**Table 2.** Effect of SiO<sub>2</sub>NPs on root and shoot Fresh Weight (FW) and root and shoot Dry Weight (DW) (in g/pot) of six test species,<sup>a</sup>

SiO <sub>2</sub> (mg L <sup>-1</sup> )	<i>Zea mays</i>				<i>Phaseolus vulgaris</i>			
	Root		Shoot		Root		Shoot	
Control (0)	FW (g pot <sup>-1</sup> )	DW (g pot <sup>-1</sup> )	FW (g pot <sup>-1</sup> )	DW (g pot <sup>-1</sup> )	FW (g pot <sup>-1</sup> )	DW (g pot <sup>-1</sup> )	FW (g pot <sup>-1</sup> )	DW (g pot <sup>-1</sup> )
400	3.96 ± 0.00 b	1.86 ± 0.00 b	7.94 ± 0.01 b	3.84 ± 0.01 b	3.60 ± 0.14 b	1.92 ± 0.02 b	5.28 ± 0.01 b	2.80 ± 0.14 b
2000	8.30 ± 0.09 a	4.23 ± 0.01 a	16.6 ± 0.09 a	6.22 ± 0.01 a	5.83 ± 0.14 a	2.70 ± 0.15 a	9.60 ± 0.04 a	4.54 ± 0.01 a
4000	3.43 ± 0.01 c	1.26 ± 0.00 c	6.87 ± 0.01 c	2.84 ± 0.01 c	2.10 ± 0.09 c	0.96 ± 0.00 c	4.54 ± 0.01 c	2.30 ± 0.09 c
	2.96 ± 0.01 d	0.90 ± 0.09 d	5.94 ± 0.01 d	1.80 ± 0.18 d	1.98 ± 0.04 c	0.40 ± 0.09 d	3.67 ± 0.00 d	0.98 ± 0.00 d

SiO <sub>2</sub> (mg L <sup>-1</sup> )	<i>Hyssopus officinalis</i>				<i>Nigella sativa</i>			
	Root		Shoot		Root		Shoot	
Control (0)	FW (g pot <sup>-1</sup> )	DW (g pot <sup>-1</sup> )	FW (g pot <sup>-1</sup> )	DW (g pot <sup>-1</sup> )	FW (g pot <sup>-1</sup> )	DW (g pot <sup>-1</sup> )	FW (g pot <sup>-1</sup> )	DW (g pot <sup>-1</sup> )
400	1.98 ± 0.00 b	0.82 ± 0.00 b	4.21 ± 0.01 a	1.72 ± 0.02 b	2.25 ± 0.00 b	1.02 ± 0.00 b	4.32 ± 0.01 b	1.54 ± 0.01 b
2000	2.42 ± 0.01 a	1.16 ± 0.00 a	4.23 ± 0.01 a	1.98 ± 0.00 a	2.32 ± 0.00 a	1.23 ± 0.01 a	5.41 ± 0.01 a	1.86 ± 0.00 a
4000	1.12 ± 0.01 c	0.11 ± 0.00 c	2.12 ± 0.01 b	0.94 ± 0.01 c	0.64 ± 0.01 c	0.12 ± 0.00 c	3.38 ± 0.00 c	1.02 ± 0.01 c
	0.30 ± 0.10 d	0.09 ± 0.00 c	1.74 ± 0.01 c	0.44 ± 0.00 d	0.62 ± 0.01 c	0.11 ± 0.00 c	2.14 ± 0.00 d	0.61 ± 0.02 d

SiO <sub>2</sub> (mg L <sup>-1</sup> )	<i>Anaranthus retroflexus</i>				<i>Taraxacum officinale</i>			
	Root		Shoot		Root		Shoot	
Control (0)	FW (g pot <sup>-1</sup> )	DW (g pot <sup>-1</sup> )	FW (g pot <sup>-1</sup> )	DW (g pot <sup>-1</sup> )	FW (g pot <sup>-1</sup> )	DW (g pot <sup>-1</sup> )	FW (g pot <sup>-1</sup> )	DW (g pot <sup>-1</sup> )
400	1.18 ± 0.01 a	0.42 ± 0.01 a	3.65 ± 0.00 a	1.24 ± 0.02 a	0.94 ± 0.00 a	0.32 ± 0.01 a	2.87 ± 0.01 a	0.84 ± 0.01 a
2000	0.98 ± 0.04 b	0.38 ± 0.00 b	3.63 ± 0.00 a	0.98 ± 0.01 b	0.62 ± 0.01 b	0.14 ± 0.01 b	1.46 ± 0.00 b	0.82 ± 0.01 a
4000	0.42 ± 0.00 c	0.10 ± 0.00 c	2.05 ± 0.02 b	0.41 ± 0.01 c	0.21 ± 0.00 c	0.08 ± 0.01 c	0.92 ± 0.01 c	0.26 ± 0.01 b
	0.11 ± 0.00 d	0.06 ± 0.00 d	1.02 ± 0.00 c	0.11 ± 0.00 d	0.18 ± 0.01 c	0.02 ± 0.00 d	0.15 ± 0.00 d	0.09 ± 0.01 c

<sup>a</sup> Values indicate mean±SE. Different lower case letters indicate significant differences (DMRT) in FW and DW (assessed separately) for each plant species across treatments ( $P \leq 0.05$ ),  $n = 4$

*retroflexus* shoot FW and *T. officinale* shoot DW.

### Effects on the Content of Photosynthetic Pigments

The results related to photosynthetic pigments for all plants are shown in Table 3. In both crops and in both medicinal plants, there was a significant increase in all photosynthetic pigments (chl *a*, chl *b*, total chl, carotenoids) when 400 mg L<sup>-1</sup> SiO<sub>2</sub> NPs were used relative to the control and 2,000 and 4,000 mg L<sup>-1</sup> SiO<sub>2</sub> NPs. For the weeds, the trend was somewhat different: in all cases, either the control or 400 mg L<sup>-1</sup> SiO<sub>2</sub> NPs significantly increased the content of all photosynthetic pigments (or 2,000 and 4,000 mg L<sup>-1</sup> SiO<sub>2</sub> NPs significantly decreased the photosynthetic pigment content).

### Effects on Total Carbohydrate, Total Protein, Free Proline and Total Amino Acid Content of Leaves

Total carbohydrates, total proteins, free proline and total amino acid content of leaves for all six plants are reported in Figures 3a-d, respectively. For all six plants, any concentration of SiO<sub>2</sub> NP significantly decreased the total carbohydrate content of leaves (Figure 3-a). However, for all six plants, 400 mg L<sup>-1</sup> of SiO<sub>2</sub> NPs significantly increased the total protein content of leaves relative to the control while higher concentrations of SiO<sub>2</sub> NPs (2,000 and 4,000 mg L<sup>-1</sup>) caused a significant decrease (Figure 3-b). Free proline content was significantly enhanced in the presence of 4,000 mg L<sup>-1</sup> SiO<sub>2</sub> NPs relative to the control and other concentrations of SiO<sub>2</sub> NPs (Figure 3-c). The total amino acid content for all six plants followed the same trend as for total protein content, with 400 mg L<sup>-1</sup> of SiO<sub>2</sub> NPs significantly increasing the amino acid content of leaves relative to the control while higher concentrations of SiO<sub>2</sub> NPs (2,000 and 4,000 mg L<sup>-1</sup>) caused a significant decrease in total amino acid content (Figure 3-d).

## DISCUSSION

Plants need 16 essential elements for growth. Although Silicon (Si) is not within this group, it is a very important component of plants, and is treated as an inorganic component (Chen *et al.*, 2000). Si is the second most abundant element in the earth's surface, makes up more than 41% of the earth's crust (Exley, 1998), and is absorbed by plants as Silicic acid [Si(OH)<sub>4</sub>]. Si promoted the number of panicles, number of spikelets/panicle, tillers, grain yield, grain filling and quality of *Oryza sativa* (Savant, 1997) stem strength in turfgrasses (Hull, 2004), the FW and DW of both roots and shoots of *Z. mays* and *Cucurbita moschata* (Liang *et al.*, 2007; Savvas *et al.*, 2009) and the number of fruits in zucchini squash (*Cucurbita pepo* L. cv. 'Rival'), therefore, increasing agricultural productivity (Savvas *et al.*, 2009). Thus, the advantageous effects of Si lie in its use by plants to enhance growth by reducing mineral toxicity, increasing resistance to biotic stresses, improving nutrient uptake, inducing a balance in plants and enhancing photosynthetic activity (Hull, 2004; Liang *et al.*, 2007). Si affected the chl content in bread wheat (*Triticum aestivum* L.) (Gong *et al.*, 2005) and total sugars, raffinose, sucrose and soluble sugars of the leaves of sugarcane (*Saccharum officinarum* L.) (Matichenkov and Calvert, 2002). It was proposed that the principal role of Si in increasing leaf chl arises from its upkeep of the chloroplast ultrastructure accompanied by an improvement of chl biosynthetic enzymes or a reduction of chl-degrading enzymes (Liang *et al.*, 2007; Savvas *et al.*, 2009).

Several studies also suggested that Si can reduce the content of protein carbonyl (oxidative proteins) and increase total soluble proteins in bread wheat (Gong *et al.*, 2005; Gong *et al.*, 2008). In addition, Si acts on mechanisms common to all plants by expressing plant signaling cascades (stress genes) as a natural defense reaction that translates into and activates a strategic signaling protein known as Mitogen-Activated Protein kinases (MAP-kinases) and Proline-Rich (PR) protein (Gong *et al.*, 2008). PR proteins cause phosphorylation of the hydroxyl group on amino acid residues by transmitting information to the nucleus (Fauteux *et al.*, 2005). Si binds to hydroxyl groups and may influence protein conformation or activity,



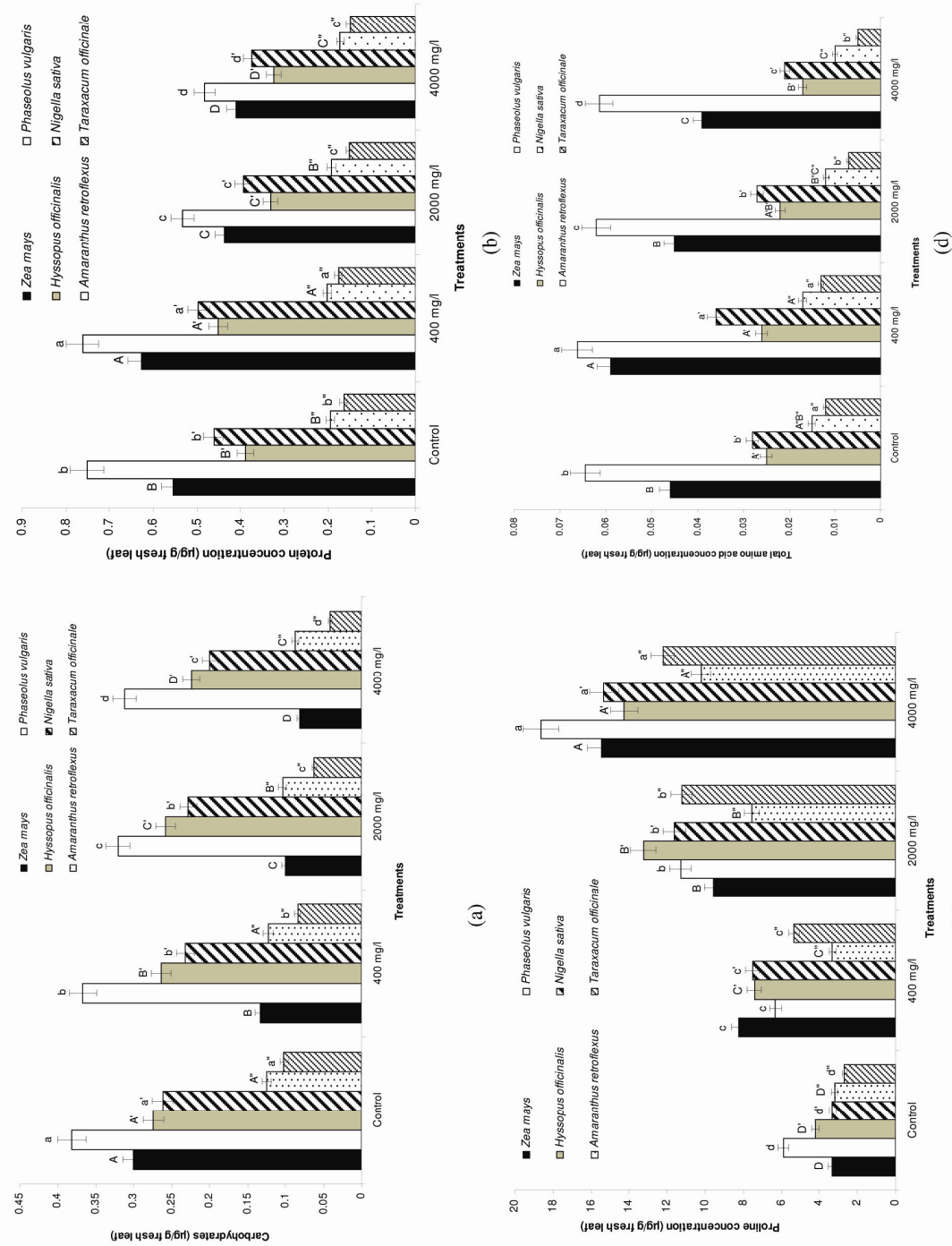
**Table 3.** Effect of SiO<sub>2</sub>NPs on photosynthetic pigments (chl *a*, *b*, *a+b*, carotenoids) (in mg g<sup>-1</sup> fresh leaf) of six test species.

SiO <sub>2</sub> (mg L <sup>-1</sup> )	Crops					
	<i>Zea mays</i>			<i>Phaseolus vulgaris</i>		
	chl <i>a</i>	chl <i>b</i>	Total chl	Carotenoids	chl <i>a</i>	Carotenoids
Control (0)	0.44 ± 0.01 c	0.36 ± 0.00 b	0.80 ± 0.02 b	0.24 ± 0.01 c	0.25 ± 0.01 b	0.45 ± 0.03 b
400	0.74 ± 0.00 a	0.43 ± 0.00 a	1.17 ± 0.00 a	0.62 ± 0.00 a	0.34 ± 0.01 a	0.90 ± 0.01 a
2000	0.49 ± 0.00 b	0.25 ± 0.00 c	0.74 ± 0.01 c	0.47 ± 0.00 b	0.28 ± 0.01 b	0.50 ± 0.02 b
4000	0.24 ± 0.01 d	0.23 ± 0.01 c	0.47 ± 0.00 d	0.26 ± 0.00 c	0.19 ± 0.00 c	0.34 ± 0.01 c
					0.15 ± 0.01 c	0.20 ± 0.00 b

SiO <sub>2</sub> (mg L <sup>-1</sup> )	Medicinal plants					
	<i>Hyssopus officinalis</i>			<i>Nigella sativa</i>		
	chl <i>a</i>	chl <i>b</i>	Total chl	Carotenoids	chl <i>a</i>	Carotenoids
Control (0)	0.34 ± 0.00 c	0.28 ± 0.01 b	0.62 ± 0.01 b	0.21 ± 0.00 c	0.38 ± 0.01 b	0.70 ± 0.02 b
400	0.62 ± 0.01 a	0.36 ± 0.01 a	0.98 ± 0.02 a	0.42 ± 0.01 a	0.58 ± 0.01 a	0.96 ± 0.01 a
2000	0.38 ± 0.01 b	0.24 ± 0.01 b	0.62 ± 0.01 b	0.32 ± 0.00 b	0.32 ± 0.00 c	0.51 ± 0.00 c
4000	0.19 ± 0.00 d	0.17 ± 0.01 c	0.36 ± 0.01 c	0.18 ± 0.01 c	0.18 ± 0.00 d	0.30 ± 0.02 d

SiO <sub>2</sub> (mg L <sup>-1</sup> )	Weeds					
	<i>Amaranthus retroflexus</i>			<i>Taraxacum officinale</i>		
	chl <i>a</i>	chl <i>b</i>	Total chl	Carotenoids	chl <i>a</i>	Carotenoids
Control (0)	0.22 ± 0.00 a	0.24 ± 0.01 a	0.46 ± 0.01 a	0.18 ± 0.01 a	0.21 ± 0.01 a	0.46 ± 0.01 a
400	0.21 ± 0.01 a	0.16 ± 0.00 b	0.37 ± 0.01 b	0.16 ± 0.01 a	0.19 ± 0.00 b	0.33 ± 0.00 b
2000	0.11 ± 0.01 b	0.10 ± 0.00 c	0.21 ± 0.01 c	0.11 ± 0.01 b	0.10 ± 0.00 c	0.19 ± 0.01 c
4000	0.04 ± 0.00 c	0.05 ± 0.00 d	0.09 ± 0.01 d	0.06 ± 0.00 c	0.06 ± 0.00 d	0.09 ± 0.00 d

Values indicate mean±SE. Different lower case letters indicate significant differences (DMRT) in photosynthetic pigments (assessed separately) for each plant species across treatments ( $P \leq 0.05$ ).  $n = 4$ .



**Figure 3.** Effect of nano-SiO<sub>2</sub> nanoparticles (400, 2000, and 4000 mg L<sup>-1</sup>) and the control (distilled water without nano-SiO<sub>2</sub>) on leaf carbohydrate content (a), on total leaf protein content (b), on leaf proline (amino acid) content (c), and leaf total amino acid content (d) in six test species. Different lower case and capital letters show significant differences (DMRT) between treatments means for any one plant species at  $P \leq 0.05$ .  $n = 4$ .



thus Si acts as a moderator and a potentiator of plant defense reactions against biotic and abiotic stresses (Fauteux *et al.*, 2005).

Studies on the effects of Si NPs on plants are limited. In fact, most studies on the benefits and risks of NPs such as TiO<sub>2</sub> (Zheng *et al.*, 2005), fullerene (Wang *et al.*, 1999), Al<sub>2</sub>O<sub>3</sub> (Yang and Watts, 2005), or zinc oxide (ZnO) (Lin and Xing, 2007) have focused on higher plants or lower plants such as fungi and algae. A common objective of all these studies was to control the uptake and subsequent effects, depending on the size of the NPs (Limbach *et al.*, 2005; Chithrani *et al.*, 2006). There are no studies on the effect of Si NPs on a wide range of plants.

The results of our study showed that 400 mg L<sup>-1</sup> SiO<sub>2</sub> NP improved seed germination in crops and medicinal plants while higher concentrations of SiO<sub>2</sub> NPs decreased seed germination, indicating its toxic effects (Figure 2). Due to the opportunistic nature of weeds, the testa may rupture more easily than that of crops and medicinal plants, thus exposing the endosperm to SiO<sub>2</sub> NP at a very early (and sensitive) stage of development, explaining why the trend for weeds would be quite different to that of the other two groups of plants examined in this study. In contrast, in weeds, no concentration of SiO<sub>2</sub> NP increased seed germination; rather, as SiO<sub>2</sub> NP concentration increased, so too did toxicity (Figure 2). E-Temsah and Joner (2012) reported that zero-valent iron NPs inhibited germination at 1,000–2,000 mg L<sup>-1</sup> in ryegrass (*Lolium perenne* L.). Lin and Xing (2007) provided new insight into the phytotoxicology of NPs (multi-walled carbon nanotubes of aluminum, alumina, zinc, and ZnO) on seed germination and root growth of six higher plants [radish (*Raphanus sativus* L.), rape (*Brassica napus* L.), ryegrass, lettuce (*Lactuca sativa* L.), maize, and cucumber (*Cucumis sativus* L.)]. Their research showed that manufactured NPs negatively impacted plant growth. More specifically, nano-Zn and nano-ZnO inhibited seed germination of ryegrass and corn at 2,000 mg L<sup>-1</sup>.

NPs have been shown to negatively affect root elongation in plants. Yang and Watts (2005) reported that 2,000 mg L<sup>-1</sup> of nano-Al<sub>2</sub>O<sub>3</sub> significantly inhibit root elongation of five plants: maize, cucumber, soybean (*Glycine max* (L.) Merr.), cabbage (*Brassica oleracea* L.) and

carrot (*Daucus carota* L.). Lin and Xing (2007) showed that nano-Al<sub>2</sub>O<sub>3</sub> was phytotoxic to maize root elongation, being reduced by 35% at 2,000 mg L<sup>-1</sup>. Salama (2012) showed that 80 and 100 mg L<sup>-1</sup> of silver (Ag) NPs significantly inhibited shoot and root elongation. At low concentrations (400 mg L<sup>-1</sup>) of SiO<sub>2</sub> NPs, root and shoot length were stimulated in crops and medicinal plants, while higher concentrations were toxic, decreasing these plant parameters; however, in weeds, all concentrations of SiO<sub>2</sub> NPs were phytotoxic (Table 1).

Mahmoodzadeh *et al.* (2013) reported that TiO<sub>2</sub> NPs affected root and shoot FW in wheat: root and shoot DW in the presence of 10 and 100 mg L<sup>-1</sup> increased these parameters but higher concentrations (1,000, 1,200, 1,500, 1,700, 2,000 mg L<sup>-1</sup>) decreased them. This study also indicated some stimulatory action of SiO<sub>2</sub> NPs on root and shoot FW and DW, at least in crops and medicinal plants, with higher concentrations being toxic (Table 2).

Zamani and Moradshahi (2013) treated red algae (*Rhodophyta*) with 0, 25, 50, 100 and 200 µM of Ag NPs (i.e., nano-Ag). High concentrations of Ag NPs (100 and 200 µM) decreased growth, total carotenoids, and total chl content after 10 days of exposure. Wei *et al.* (2010) treated turpin (*Scenedesmus obliquus* L.) with 0, 25, 50, 100 and 200 µM of silica NPs. When treated with 25 µM of silica NPs, photosynthetic pigments (chl *a/b* and carotenoids) increased as NP concentration increased, but decreased after 96-hours exposure. Jiang *et al.* (2012) reported that Ag NPs and AgNO<sub>3</sub> significantly reduced plant chl *a/b* content. Unlike all these studies, our study shows that 400 mg L<sup>-1</sup> SiO<sub>2</sub> NPs significantly improved the content of all photosynthetic pigments for all six test species (Table 3), confirming the findings for other studies in which low and moderate levels of NPs increased photosynthetic pigments while high concentrations had a negative impact. Nitrogen and phosphorus are essential elements for plants as they are related to cell division and growth, and treatment with metals decreased their uptake (Batty and Younger, 2003). These elements can affect the synthesis of photosynthetic pigments (Batty and Younger, 2003), so a decrease in chl could be related not only to increased degradation but also to decreased synthesis. Reduced content of

photosynthetic pigments and inhibited photosynthetic activity may be related to a decrease in access to light due to the accumulation of SiO<sub>2</sub> NPs on the surface of plant cell walls, causing a shading effect (Wei *et al.*, 2010).

Salama (2012) reported that 60 mg L<sup>-1</sup> of Ag NPs increased carbohydrate content in *P. vulgaris* and *Z. mays* (57 and 62% more than the control) and that at 80 and 100 mg L<sup>-1</sup> of Ag NPs, carbohydrate concentration was reduced (19 and 18% for common bean and 28 and 31% for maize relative to the control). Our results show that in field crops, medicinal and weed plants, carbohydrate concentration decreased significantly as SiO<sub>2</sub> NP concentration increased, suggesting the toxic nature of SiO<sub>2</sub> NPs over a wide range of plants. Photosynthesis is a plant's main metabolic pathway in which sugars are synthesized from CO<sub>2</sub>, water, and light energy. These sugars or carbohydrates serve as the origin of energy for a plant's other metabolic procedures. Thus, a low level of photosynthetic activity caused by SiO<sub>2</sub> NP stress can decrease carbohydrates directly, causing plant growth to be reduced.

Our results showed that with increasing SiO<sub>2</sub> NP concentration, there was a decrease in total protein content, with maximum values at 400 mg L<sup>-1</sup> SiO<sub>2</sub> NPs (Figure 3-b). At 60 mg L<sup>-1</sup>, Ag NPs increased the protein content of *P. vulgaris* and *Z. mays* leaves (30 and 24%, respectively, more than the control) while 100 mg L<sup>-1</sup> significantly decreased the protein content (32 and 18%, respectively, less than the control) (Salama, 2012).

As SiO<sub>2</sub> NP concentration increased, proline content increased for all six crops (Figure 3-c). Proline accumulates in plant under a wide range of stress conditions such as high and low temperature, pathogen infection, anaerobiosis, nutrient deficiency, heavy metal toxicity, UV-irradiation, atmospheric pollution, salinity, water deficiency, high light intensity and extreme temperatures (Mansour, 2000). Proline accumulation in plant tissues has been proposed to result from a reduction in proline degradation, an intensification of proline biosynthesis, or a reduction in proline utilization or in the synthesis and hydrolysis of proteins. It protects plants under stress by stabilizing cell membranes by interacting with phospholipids, and by protecting

folded protein structures against denaturation, functioning as a free radical scavenger, or serving as a source of energy and nitrogen.

As SiO<sub>2</sub> NP concentration increased, total amino acid content decreased (Figure 3-d). Amino acids are the precursors or activators of phytohormones and growth substances. Glycine and glutamic acid are fundamental metabolites in the formation of plant tissue and chl synthesis. These amino acids raise the chl content in plants which increases the absorption of light energy and leads to increased photosynthesis. As a result, if the amino acids content decreases, photosynthetic pigments, total protein, total carbohydrate content, and other related metabolic problems in plants can also be reduced.

## CONCLUSIONS

The use of SiO<sub>2</sub> NPs at high concentrations can result in toxic effects on morphological, physiological, and biochemical characteristics of crop, medicinal and weed plants while, in select cases and for select parameters, a lower concentration (400 mg L<sup>-1</sup>) can in fact be beneficial. Thus, caution is urged in the use and disposal of such materials into the environment. Regarding the toxic levels, future studies should focus on levels of uptake and retention, source-sink relations and the localization of SiO<sub>2</sub> NP sinks, the mechanism of phytotoxicity and uptake kinetics, and interactions within cells. However, since lower concentrations present positive aspects, concentrations of SiO<sub>2</sub> NPs should be optimized for each crop (a narrower range of 10-1,000 mg L<sup>-1</sup> should be tested next) in a bid to maximize yield and other ergonomically favorable factors. Ideally, toxicity tests should be conducted hand in hand with optimization trials.

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واکنش های مورفولوژیکی، فیزیولوژیکی و بیوشیمیایی گیاهان زراعی ( *Zea mays* L., *Phaseolus vulgaris* L.), گیاهان دارویی ( *Hyssopus officinalis* L., *Nigella sativa* L.), و علف های هرز ( *Amaranthus retroflexus* L., *Taraxacum officinale* F. H. Wigg) در معرض نانوذرات  $\text{SiO}_2$

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### چکیده

در این مطالعه، گیاهان زراعی (*Zea mays* L., *Phaseolus vulgaris* L.)، دارویی (*Hyssopus officinalis* L., *Nigella sativa* L.) و علف هرز (*Amaranthus retroflexus* L., *Taraxacum officinale* F. H. Wigg) به طور جداگانه با سه غلظت نانوذرات  $\text{SiO}_2$  (۴۰۰، ۲۰۰۰ و ۴۰۰۰ میلی گرم / لیتر) تحت تیمار قرار گرفتند. اثرات این تیمارها بر خصوصیات مورفولوژیک و بیوشیمیایی از جمله جوانه زنی، طول ساقه و ریشه، وزن تر ریشه و ساقه، وزن خشک ریشه و ساقه، رنگدانه های فتوسنتزی، کربوهیدرات کل، پروتئین کل، اسید آمینه کل و آمینو اسید پرولین مورد بررسی قرار گرفتند. در گیاهان زراعی و دارویی، در غلظت ۴۰۰ میلی گرم بر لیتر نانو ذره جوانه زنی بذری، طول ریشه و ساقه، وزن تر ریشه و ساقه (به جز *H. officinalis*)، وزن خشک ریشه و ساقه، رنگدانه های فتوسنتزی، پروتئین کل و اسید آمینه کل (به جز *H. officinalis*) به طور قابل توجهی افزایش یافته است. در علف های هرز، با افزایش غلظت نانو ذره از ۴۰۰ تا ۴۰۰۰ میلی گرم بر لیتر، جوانه زنی، طول ریشه و ساقه، وزن تر ریشه و ساقه، وزن خشک ریشه و ساقه، رنگدانه های فتوسنتزی و همچنین پروتئین کل کاهش یافته است. کربوهیدرات کل در تمام گیاهان به طور قابل توجهی به جز *A. retroflexus* در ۴۰۰ میلی گرم بر لیتر نانو ذره کاهش یافته است. در تمام گونه های گیاهی، با افزایش غلظت نانو ذره  $\text{SiO}_2$ ، محتوای پرولین به طور قابل توجهی افزایش یافته است. غلظت های پایین تر نانو ذره  $\text{SiO}_2$  می تواند اثرات مفیدی بر روی خصوصیات مورفولوژیک، فیزیولوژیکی و بیوشیمیایی گیاهان داشته باشد.