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ORIGINAL ARTICLE

Evaluating SiO_2 Nanoparticles Effects on Developmental Characteristic and Photosynthetic Pigment Contents of *Zea mays* L.

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ABSTRACT

The usage of the nanoparticle is increasing in different fields, but till yet, very limited studies have been performed as far as consequence of nanomaterials and plant interactions is concerned. In this study, the effects of silica nanoparticles (SiO_2) on developmental stages of Zea mays L. viz. seed germination, rate of root and stem elongation, relative water content (RWC) and photosynthetic pigment content have been investigated. The results exhibited that exposure to the silica nanoparticles at 0, 400, 2000, and 4000 mg/L concentrations could significantly ($p<0.05$) increase the root elongation and seed germination in comparison to control. In addition, shoot elongation, relative water content and photosynthetic pigment content, differed between treated and control samples.
Keywords: *Developmental characteristic, SiO_2 Nanoparticles, Photosynthetic pigment, Zea mays L.*

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INTRODUCTION

Nanoparticles are exploring a wide range of applications in biomedical sciences, drug delivery, gene therapy, cell targeting, magnetics, optics, mechanics, catalysis and energy science [1-4]. In the past few years, nanomaterials and nanotechnology have been extensively used in various industry and commerce areas in many countries. Nanoparticles are atomic or molecular aggregates with at least one dimension between 1 and 100 nm, contrastingly, nanomaterials are great and versatile group of materials in which one or several dimensions are of size 1-100 nm [5]. This size can significantly change their properties compared to the bulk material [6]. Nanotechnology has an ability to improvise the agricultural and food industry with new tools like instant disease detectors and increasing plant's ability to absorb nutrients [7]. The exclusive properties of these materials, such as a large specific surface area and greater reactivity, have raised questions concerning potential adverse effects on human and environmental health [8]**.** In spite of the extensive growth of nanotechnology and nanomaterials through the last 20 years, the recent focus has been turned on to the potential toxicological effects on humans, animals, and the environment through the exposure of metal nanomaterials. Until now only a very limited number of phyto and eco-toxicological studies have been performed. The results of these studies have been reported with an aim to supply more insight into the correlations between plants and nanomaterials. At present there is an extensive discussion in relation to the risks and benefits of the many nonmaterial into the environment [9]. Because of the widespread utilization of these products it is expected that nanoparticles will soon find their way into aquatic, terrestrial and atmosphere environments, where their fate and behavior are largely unknown**.** Therefore, it demands research related risk assessment evaluation in order to clarify all related aspects of the concern, but risk assessment is quite a difficult task for nanomaterials since an insignificant research has been carried out. Moreover, there are many unknown effects that nanomaterials have on ecosystems. The aim of this study is evaluating the effect of SiO_2 nanoparticle on *Zea mays* L. growth.

MATERIALS AND METHODS

Nanoparticle

The nanosilicon dioxide was procured from Nanoamor Co., Iran with the specifications shown in Figure 1 and Table 1. Average particle size of experimental nanoparticle was 20 nm and purity was 99.5% (Table 1). The shape of SiO_2 nanoparticles was spherical (Fig 1). The nanoparticles were dispersed in distill water at four concentrations *viz.* 0, 400, 2000 and 4000 mg/L and then sterilized at 120°C for 30 minutes. Ultrasonicator was used for easy dispersion of nanoparticles without precipitation. There was sufficient shaking to break up agglomerates. The treatment concentration for each nanoparticle was prepared separately, devoid of dilutions. The particles were weighed and dispersed in water. The nanoparticles suspensions were dispersed under suction for 20 minutes before use.

Seeds

Seeds of *Zea mays* L. were surface sterilized in 5% (w/v) sodium hypochlorite with 20 minute incubation period. After three washes with distilled water, the seeds were germinated on wet filter paper in sterile Petri dishes. Corn seeds were grown hydroponically, in solution with known concentrations of SiO_2 nanoparticles. The growth of plants was visually observed and recorded (Fig 2). Germination percentages were determined by comparing the numbers of seeds that developed a primary root of at least 1 mm to the total number of seeds planted in each dish. Relative root growth inhibition was calculated as the difference between the average primary root length of the unexposed control plants and treatment plants, average root length divided by the primary root length of the control.

Relative water content (RWC) measurement

To determine the relative water content of different parts of the plant, the leaf samples were removed and their fresh weight (FW), dry weight (DW) and turgid weight (TW) was determined. Leaf relative water content, was measured by soaking leaf sample (0.5 g) in 100 ml of distilled water at 4°C in the dark for 24 h. The turgid leaves were quickly blotted dry prior to the turgid weight measurement. Dry weight of leaves was determined after oven-drying at 70°C for 48 h. The relative water content (RCW) was determined by subtracting fresh weight from dry weight and multiplying this number by the difference between turgid weight and dry weight. RWC was calculated according to Smart and Bingham [10], using the following equation:

$$RWC = [\text{fresh weight- dry weight/ turgid weight} - \text{dry weight}] \times 100$$

Photosynthetic Pigment Measurement

The content of photosynthetic pigments was determined according to the method of Lichtenthaler and Wellburn [11]. Two hundred mg of leaf tissue was weighed and powdered using liquid nitrogen. After adding 80% acetone, the volume was brought to 25 ml. The resulting solution was centrifuged at 4800 rpm for 20 min. The supernatant was used for measurement of chlorophyll a, b and the carotenoids. Observance of the clear supernatant was read at 663.2, 646.8 and 470 NM (Shimadzo spectroscopy, A160 model, Japan) and pigment concentrations were calculated using the following formula:

$$\text{Chl a } (\mu g/ml) = 12.25 * A_{663.2} - 2.79 * A_{646.8}$$

$$\text{Chl b } (\mu g/ml) = 21.5 \times A_{646.8} - 5.1 \times A663.2$$

$$\text{Chl a + Chl b } (\mu g/ml) = 7.15 \times A_{663.2} + 18.71 \times A_{646.8}$$

$$B\text{-carotene } (\mu g/ml) = (1000 * A_{470} - 1.82 * Ca - 85.02 * Cb) / 198$$

In this formula Chl a, Chl b, Chl T and CX+C are, chlorophyll a, chlorophyll b, total chlorophyll and carotenoid concentrations respectively.

Statistical Analysis

The experimental designs were randomized complete block and each value reported is the average of three replicates. The raw data were imported into Microsoft Excel 2007 program for calculations and graphical representation. SPSS (version 11.5) software was used for analysis of variance. Quantitative changes of different parameters were analyzed through analysis of variance (ANOVA), with Duncan's multiple range tests at $P<0.05$ being used to determine significant differences among treatments. All consequences are presented as the mean ± standard deviation (SD). The result was considered significant if $P<0.05$, when compared to the control.

RESULTS AND DISCUSSION

Effect of SiO_2 Nanoparticles on Seed Germination

Based on experimental results, it was proclaimed that seeds uptake the SiO_2 nanoparticles from the hydroponic solution. This study concluded that the nanoparticles enhanced the growth of the seed resulting in longer root elongation compared to a control group (Fig 3). By increasing the SiO_2 nanoparticles concentration, seed germination elevated at 400 mg/L but decreased at 2000, and 4000 mg/L respectively.

Effect of SiO_2 Nanoparticles on Root and Shoot Elongation

SiO_2 nanoparticles enhanced the growth of the root and seed elongation when compared to a control group (Fig 4). The result shows that with increasing concentration of nanoparticles at 0 to 4000 mg/L, size of largest root and mid-spaces of two root lengths increased but the size of the shoot decreased. This result illustrated that SiO_2 nanoparticles have positive effects on root and negative effects on shoot elongation.

Effect of SiO_2 nanoparticles on Plant RWC

Relative water content (RWC) is an index demonstrating the amount of water in the plant organs and shows the ability of a plant in maintaining water under stress conditions. Therefore, in an experimental controlled environment, the measured RWC clearly showed the response of a plant. The higher the measured amount, the greater the ability of the treatment to preserve water [12]. RWC in plant decreased by SiO_2 nanoparticles at 400 mg/L, 2000 mg/L and 4000 mg/L concentrations respectively in comparison to control sample, but this decrease were low in 4000 mg/L concentration. Total weight, weight of root, stem and leaf and weight of root (Fig 5), dry weight of leaf, stem and root (Fig 6), wet weight of leaf, stem and root (Fig 7) and relative water content estimates of *Z. mays* treated by SiO_2 nanoparticles at four concentrations; 0, 400, 2000, and 4000 mg/L (Fig 8) respectively, is demonstrated.

The Effect of SiO_2 Nanoparticles on Photosynthetic Pigment

The effect of SiO_2 nanoparticles on photosynthetic pigment contents is shown in Fig 9. It evidenced the effect of SiO_2 nanoparticles on the contents of chlorophyll a, b and carotenoids. However, nanoparticles at 400 mg/l, 2000 and 4000 mg/L concentrations caused an increased content in all the photosynthetic pigments in *Z. mays* in relation to the control. This increase was comparatively more in 400 mg/l concentration.

CONCLUSION

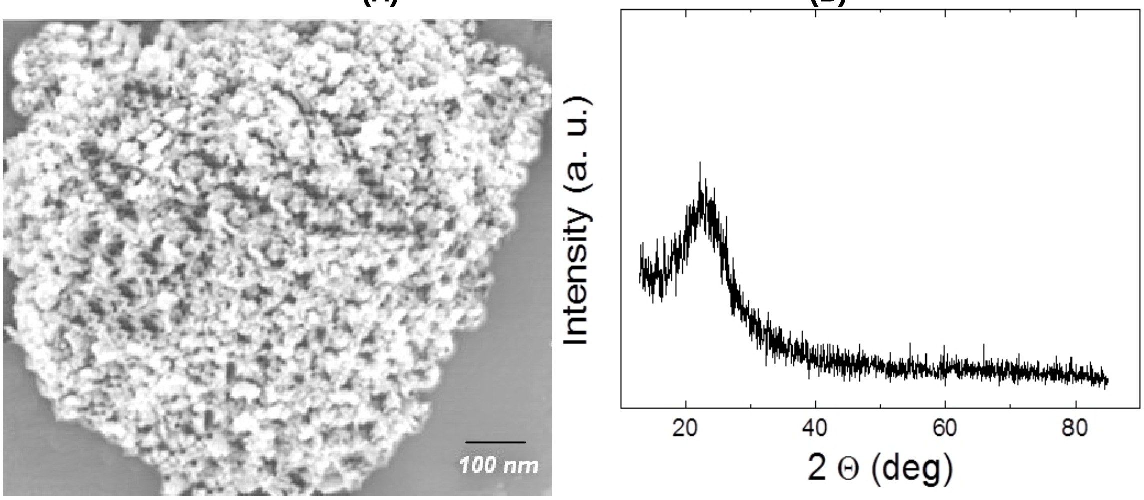
The present experimental study was focused on the potential effect of engineered nanoparticles on *Z. mays* L. plant. The growth rates of the plant roots were enhanced as a result of exposure to SiO_2 nanoparticles (Fig 4). As far as seed germination is concerned, with increase in concentration of SiO_2 nanoparticles, the seed germination increased at 400 mg/L but lowered in 2000 and 4000 mg/L. Prior studies have provided similar reports where positive effects of NPs on germination and growth of plants have been authenticated. For example, TiO_2 and SiO_2 NPs were found to enhance not only the germination but also the growth of Glycine max seeds [13]. Similarly, carbon nanotubes (CNT) were learned to progress germination and root elongation of tomato seeds [14]. The emergence of such observations is possibly due to an enlarged water uptake by seeds in the presence of high concentrations of NPs [15]. Till yet nil studies were found describing the mechanism by which SiO_2 nanoparticles affect the uptake, translocation and growth of plants. In the present study, RWC in plant decreased by SiO_2 nanoparticles at 400 mg/L, 2000 mg/L and 4000 mg/L concentrations as compared to control sample. Low decrease in 4000 mg/L concentration can be probably due to unusual increases of root growth in this concentration. Until now no studies were explored explaining the effects of nanoparticles on plants. Plants can efficiently utilize the absorbing light energy when treated with SiO_2 nanoparticles and it is recommended that an increase in photosynthetic pigment is a natural response of plants. Nanoscale SiO_2 increased leaf chlorophyll a, b and carotenoid content in treated plants irrespective of concentrations as compared to control. SiO_2 nanoparticles at 400 mg/L recorded the highest chlorophyll a, b and carotenoid content (Fig 9). Higher chlorophyll accumulation is caused by matching effect of other intrinsic nutrients like magnesium, iron and sulfur. Similar results were experienced by Zhang *et al*., [16] where *Spinacia oleracea* seeds were treated with TiO_2 nanoparticles. This is in concurrence with our results wherein in 2000 mg/L and 4000 mg/L concentrations of SiO_2 nanoparticles, the rate of increase in chlorophyll a, b and carotenoids content, reduced. It can be safely said that exposure to higher concentrations of nanoparticles may create some damages in plant photosynthetic apparatus. Till date, there are few studies reported on the phytotoxicity effects of nanoparticles on plants. The toxic effects considerably depend on many factors viz. the type of nanoparticles, plant species, and the stage of plant development that should be surveyed in future studies. Therefore it can be safely said that yet, there are many gaps in our understanding of the toxicity of nanoparticles and there are several unresolved problems and new challenges as far as the biological effects of these nanoparticles is concerned.

Table 1 Detail of Nano SiO_2 used in Present Study (Stock #: 4860MR) (A and B).

Components	Contents (%)
Al	0.001
Fe	0.001
Sr	0.004
Ca	0.002
Mg	0.001
Cl	0.001
Cr	0.004

Nano Material	Silicon Dioxide (Siox, x=1.2-1.6, amorphous)
Purity	99.5%
APS	20 nm
SSA	160 m^2/g
Color	White
Morphology	Spherical
Bulk density	0.08 g/cm^3
True density	2.2-2.6 g/cm^3

(A) (B)



(A) (B)

Fig. 1 Transmission Electron Microscopy (TEM) image of SiO_2 nanoparticles (A), X-Ray Diffraction Image of SiO_2 Nanoparticles (B).

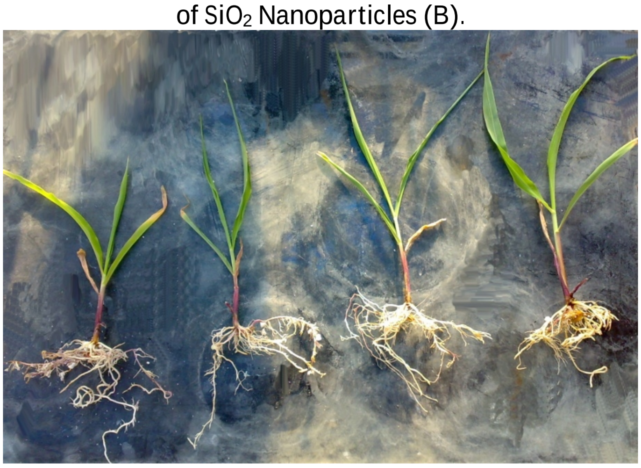


Fig. 2 *Z.mays* after 21 days of planting. Right to left treated with 0 (control), 400, 2000, and 4000mg/L concentrations respectively.

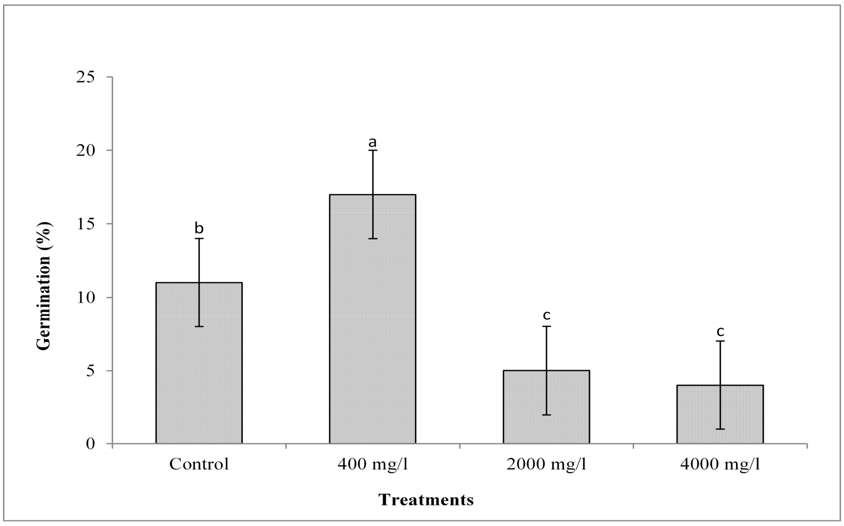


Fig. 3 Effects of varied concentrations of SiO_2 nanoparticles on seed germination. Different lower case and capital letters show significant differences between means at *P* ≤ 0.05.

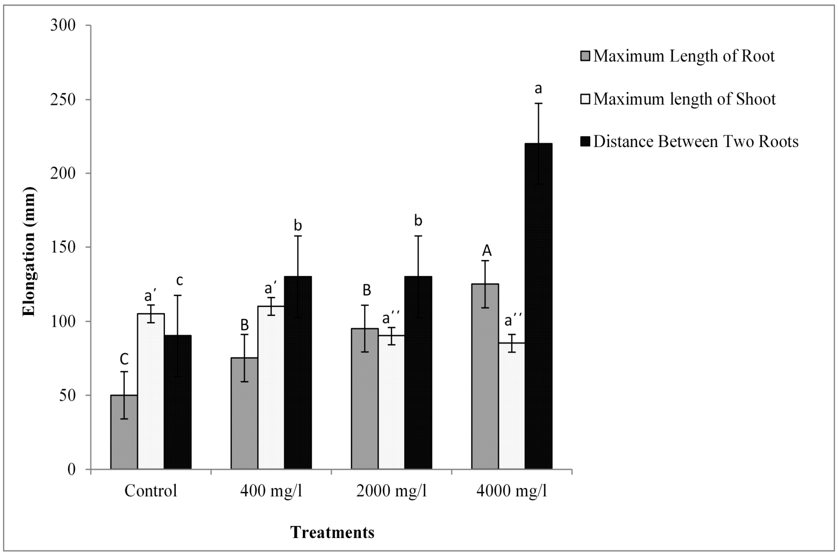


Fig. 4 Effects of varied concentrations of SiO_2 nanoparticles on root and shoot elongation. Different lower case and capital letters show significant differences between means at *P* ≤ 0.05.

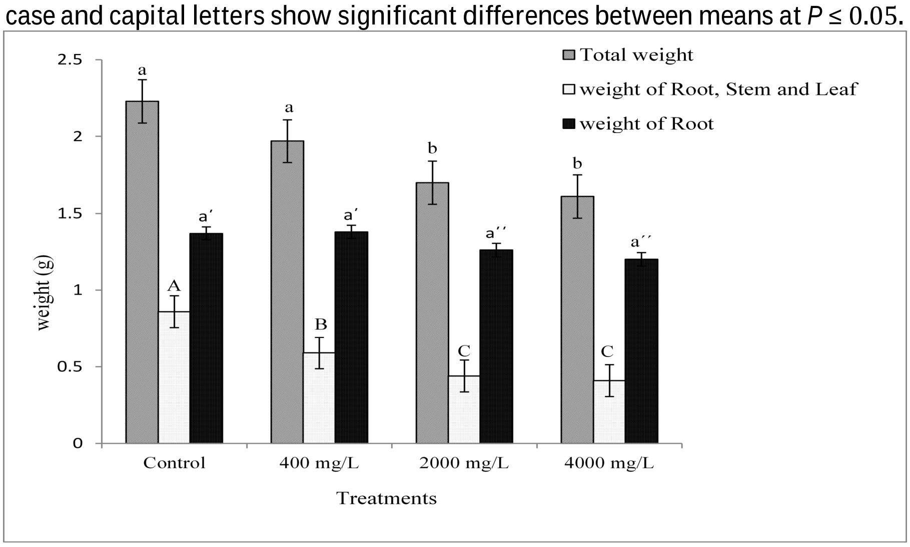


Fig. 5 Effects of varied concentrations of SiO_2 nanoparticles on total weight, weight of root, stem, leaf and weight of root. Different lower case and capital letters show significant differences between means at *P* ≤ 0.05.

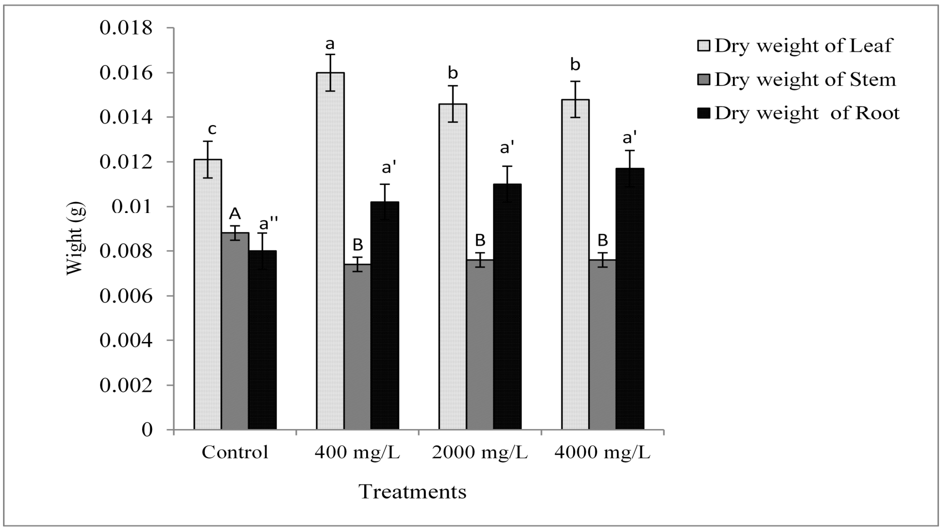


Fig. 6 Effects of varied concentrations of SiO_2 nanoparticles on dry weight of leaf, stem and root. Different lower case and capital letters show significant differences between means at *P* ≤ 0.05.

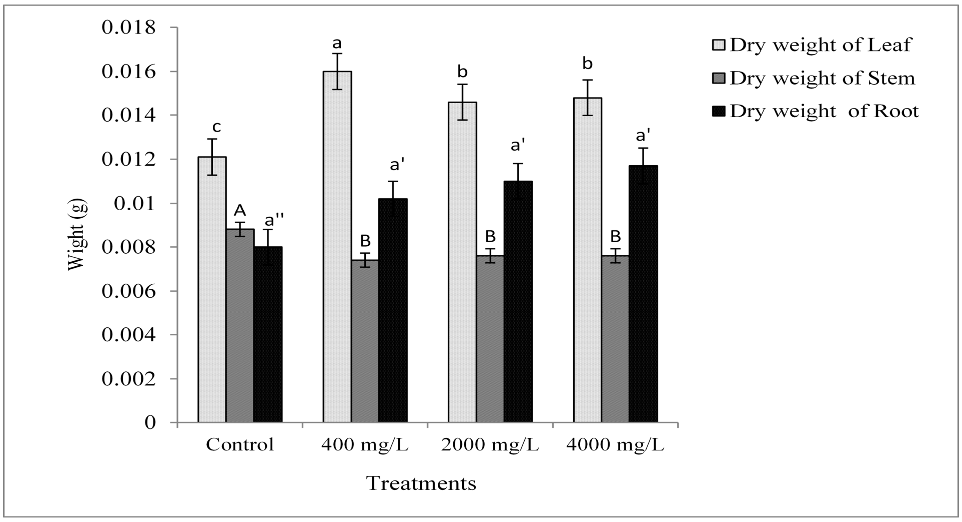


Fig. 6 Effects of varied concentrations of SiO_2 nanoparticles on dry weight of leaf, stem and root. Different lower case and capital letters show significant differences between means at *P* ≤ 0.05.

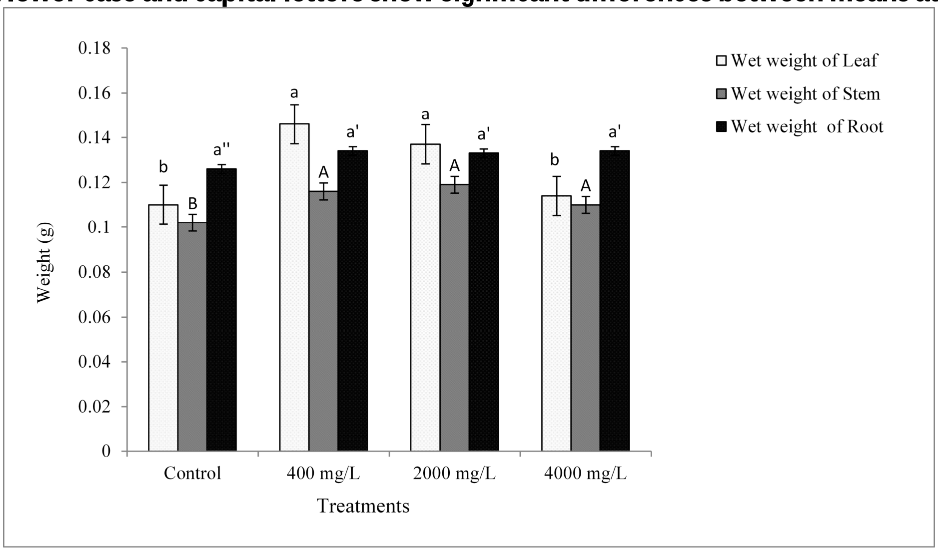


Fig. 7 Effects of varied concentrations of SiO_2 nanoparticles on wet weight of leaf, stem and root. Different lower case and capital letters show significant differences between means at *P* ≤ 0.05.

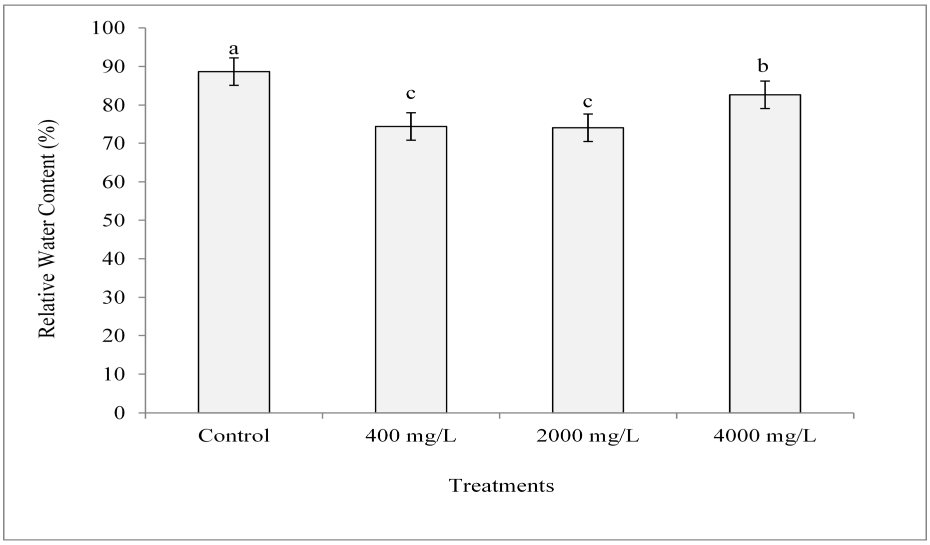


Fig. 8 Effects of varied concentrations of SiO_2 nanoparticles on relative water content. Different lower case and capital letters show significant differences between means at $P \leq 0.05$

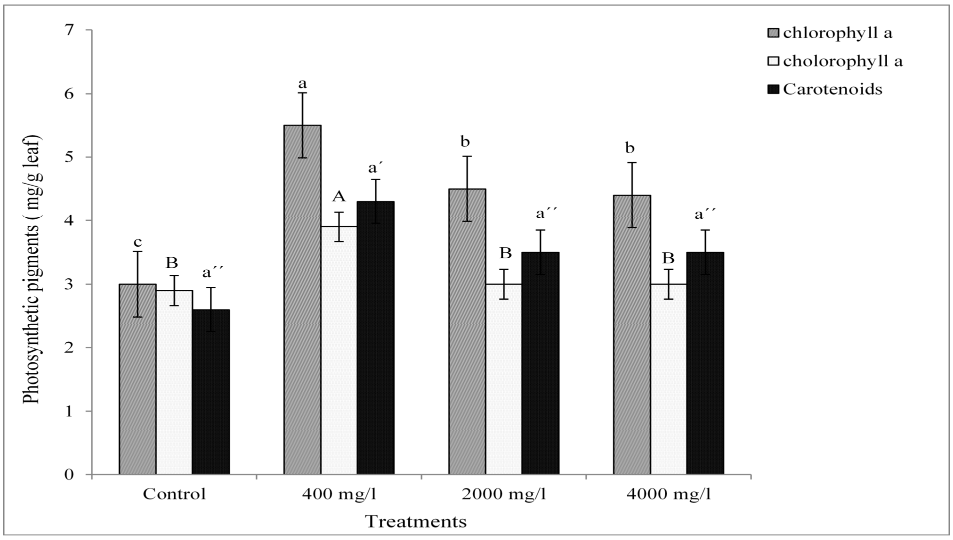


Fig. 9 Effects of varied concentrations of SiO_2 nanoparticles on photosynthetic pigments. Different lower case and capital letters show significant differences between means at $P \leq 0.05$.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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