# Effect of $SiO_2$ nanoparticles on phytochemical and anatomical alterations in *Anthemis gilanica*

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## Abstract

In this research, effects of $SiO_2$ nanoparticles (NPs) on the growth, antioxidant properties, and phenolic and flavonoid contents were investigated in *Anthemis gilanica* plants from the Asteraceae family. Following seed germination, seedlings were cultured under Hoagland growth media and were treated with different concentrations of $SiO_2$ NPs (0, 2, 4, 6, and 8 $gl^{-1}$). Results showed that $SiO_2$ NPs significantly enhanced relative water content (RWC), dry and fresh weights, and shoot length especially at 4 $gl^{-1}$, but with increasing concentrations of NPs root length decreased. Xylem number and size and also stele diameter increased up to 6 $gl^{-1}$, and then decreased at higher concentrations. Total phenol and flavonoid contents increased under different concentrations of $SiO_2$ NPs, and the maximum content was observed at 6 $gl^{-1}$. Moreover, $SiO_2$ NPs increased antioxidant activity of the extracts by reducing $IC_{50}$ content especially at 6 $gl^{-1}$. In conclusion, the study proposes that $SiO_2$ NPs can improve growth and bioactive compounds in *A. gilanica* by induction of anatomical alterations.

**Keywords:** *Anthemis gilanica*; flavonoid; growth parameters; phenol, $SiO_2$ nanoparticles

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## Introduction

*Anthemis gilanica* (*A. gilanica*) is a well-known medicinal plant species from the Asteraceae family that is distributed in the north of Iran (Houshmand et al., 2012). Asteraceae species are the rich source of natural antioxidant which is traditionally used in folk medicine (Deans et al., 1990), and is used in pharmaceuticals, cosmetics, aromatherapy, perfumery, and food industries (Eser et al., 2017). Various species of the genus *Anthemis* have been used in the treatment of gastrointestinal disorders, stomachache, hemorrhoids, earaches, and deafness (Eser et al., 2017). The main chemical constituents of *Anthemis* include terpenoids, phenolic and

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flavonoid compounds, flavone derivatives, and carotenoids that have antibacterial properties and antioxidant activity in neutralizing free radicals (Eser et al., 2017; Jafari et al., 2013). Plant phenolics are biosynthesized biogenetically via the shikimate/phenylpropanoid pathway and play an important role as chemopreventive agents. These compounds act as effective free radical scavengers in cells. The antioxidant potential of phenolic compounds in biological systems is dependent on the reduction potentials of the radicals (Chutipaijit et al., 2009). These compounds have been found to have many therapeutic properties including as cardioprotective antimicrobial, anticancer, and anti-inflammatory agents and also in improving the immune system, making them an appropriate candidate for medicinal and pharmaceutical applications (Sulaiman and Balachandran, 2012).

Nanoparticles (NPs) have multifunctional properties with broad application in various fields such as agriculture, medicine, nutrition, energy, and plant sciences (Chandran et al., 2006). Silica (Si) is a non-essential element that can induce biotic and abiotic stress tolerance in plants (Suriyaprabha et al., 2013a). It is known as one of the most beneficial elements that improves plant growth and development through improving plant water status, activating defensive compounds and enzymes, modifying ultrastructure of leaf organelles, and reducing reactive oxygen species (Karimi and Mohsenzadeh, 2016; Luyckx et al., 2017). $SiO_2$ NPs have been regarded as the nanoform (<100 nm) of silicon dioxide which can affect plant growth and antioxidant enzymes activities (EL-Kady et al., 2017). $SiO_2$ NPs application caused a significant increase in photosynthetic pigments content in *Zea mays* (Karimi and Mohsenzadeh, 2016). The exogenous application of $SiO_2$ NPs significantly induced the shoot and root dry weights in rice and wheat seedlings (Imtiaz et al., 2016). $SiO_2$ NPs also induced phytochemical constituents and antioxidant activity in *Matricaria chamomilla* seedlings. There is scant information about the effects of $SiO_2$ NPs on phytochemical constituents and antioxidant activity in medicinal plants, and until now such has not been investigated on the *in vitro* plants of *A. gilanica.* Thus, the objective of this study was to evaluate the impact of different concentrations of $SiO_2$ NPs on growth, antioxidant properties, and phenolic compounds of *A. gilanica*.

## Materials and Methods

### Plant material and culture conditions

Seeds of *A. gilanica* were collected from Gilan Province (Rodsar city and north of Iran). The collected species was identified at the Department of Pharmaceutical Chemistry of Tehran Medicinal Sciences, Islamic Azad University and saved as *A. gilanica* Bornm. and Gauba with 603_AUPF voucher number.

Germination of *A. gilanica* was performed by sowing seeds in Tref peat in a greenhouse under a 15 h light/9 h dark photoperiod at 27 ± 2 °C temperature. After two weeks, the seedlings were transplanted to perlite in plastic pots. Each pot was considered as one replicate and there were four replicates of each treatment. Each pot was treated with different concentrations of $SiO_2$ NPs (0, 2, 4, 6, and 8 $gl^{-1}$) with 100 ml of half strength Hoagland nutrient solution (pH 6.8 - 7) (Hoagland and Arnon, 1950). A foliar spray of $SiO_2$ NPs (once a week) was applied to the plants at vegetative stage. The final harvesting was done 4 weeks after the start of treatments, and then leaves and roots were obtained. Five plants per treatment were used for analyses in all the experiments. These samples were then oven-dried at 60 °C for 3 days and the dry weight was determined.

### Growth parameters

Fresh and dry weights were measured as the growth parameters. Relative water content (RWC) of fresh leaves was determined according to Whetherley (1950) and was calculated using the following equation:

$$RWC\ (\%) = [(FW- DW)/ (SW- DW)] \times 100$$

Water-saturated weight (SW) of the plants was estimated by immersing samples in ultra-pure water at 4 °C in the dark for 24 h, and dry weight was obtained after oven drying (40 °C for 72 h) until a constant weight was achieved.

### Anatomical study

Stems of all treated samples were fixed in formalin–alcohol–glacial acetic acid (1:18:1% v/v). Cross-sections were made at the middle of stems (2 cm above the root). Stem cross-sections were then stained with Methyl Green and Bismarck Brown colors (Nourbakhsh et al., 2008). Sections were observed under a microscope (Nikon, E200, Japan), and images were captured by a digital camera (Nikon, Cooplix S10 Model, Japan).

## Total phenol and flavonoid content

Leaf dried samples (2 g) were extracted with methanol (80%) and then centrifuged at 5000 rpm for 20 min. The supernatant was used for quantification of total phenolics, flavonoids, and DPPH activity.

Total phenol content was determined by using Folin–Ciocalteu reagent based on Vermerris and Nicholson (2006). Then, 0.1 ml of methanolic extract was mixed with 2.5 ml Folin–Ciocalteu reagent. Then sodium bicarbonate (7%) solution was added to the mixture. The mixtures were incubated for 1 h before the absorbance was recorded at 765 nm. Gallic acid was used as a standard for the construction of calibration curve of phenolic.

Total flavonoid contents were determined using the aluminum chloride colorimetric method (Chang et al., 2002) with some modification. In brief, 0.5 ml of extract was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate, and 2.8 ml of distilled water, and the mixture was kept at room temperature for 30 min. The absorbance reading was taken at 415 nm and routine was applied as a standard.

## DPPH activity

The free radical scavenging effects of the extracts were determined based on reducing the 2, 2-diphenyl-2-picryl hydrazyl (DPPH) solution. The sample (one ml, 50–300 µg $ml^{-1}$) was mixed with 2.5 ml of 0.5 mM methanolic solution of DPPH. The absorbance of the solution was reported by a UV–visible spectrophotometer at 517 nm at room temperature after 30 min in darkness. The free radical scavenging activities of the solutions was expressed as IC50 (µmol $ml^{-1}$). The inhibitory concentration ($IC_{50}$) of the seedlings required to inhibit 50% of the DPPH radicals obtained from the standard curve was compared to that of standard antioxidants (ascorbic acid 50-300 mM). The free radical scavenging activity was determined as:

$$(\%) = 100\ (A - B)/A$$

where A is the absorbance of the DPPH solution and B is the absorbance of the sample reaction mixture (Patro et al., 2005).

## Statistical Analysis

All assays were performed in four replicates and presented as mean ± standard error. Statistical analysis was carried out using a least significant difference test with one-way analysis of variance (ANOVA) and a Duncan test using the SPSS-21 Version. The statistical significance was considered at $p \leq 0.05$.

## Results

The results of growth parameters measurement under different concentrations of $SiO_2$ NPs are depicted in Table 1. Our study revealed significant effects of various concentrations of $SiO_2$ NPs on the dry and fresh weights of *A. gilanica* shoots and roots. The dry and fresh weights of *A. gilanica* seedlings' root at 4 and 6 g $l^{-1}$ concentrations of $SiO_2$ NPs showed significant increase compared with the control. However, at 2 and 8 g $l^{-1}$ of $SiO_2$ NPs, the fresh and dry weights of root did not significantly change compared with the control. Shoot fresh weight in *A. gilanica* seedlings increased significantly at 4 and 6 g $l^{-1}$ of $SiO_2$ NPs and did not change significantly at 2 and 8 g $l^{-1}$ of $SiO_2$ NPs compared with the control. The $SiO_2$ NPs application had a significant effect on the dry weight of the *A. gilanica* seedlings shoot. The increases in dry weight of shoots at 4, 6, and 8 g $l^{-1}$ of $SiO_2$ NPs were 124.07%, 75.92%, and 50.92%, respectively, as

Table 1
Effect of different concentrations of $SiO_2$ NPs (0, 2, 4, 6, and 8 g $l^{-1}$) on fresh and dry weights of root and shoot, root length, shoot length, and relative water content (RWC) of *A. gilanica* seedlings; values are a mean of four experiments ± SE. Different letters indicate significant differences at$p \leq 0.05$.

| $SiO_2$ NPs treatment (g $l^{-1}$) | Shoot dry weight (g) | Root dry weight (g) | Shoot fresh weight (g) | Root fresh weight (g) | RWC (%) | Shoot length (cm) | Root length (cm) |
|---|---|---|---|---|---|---|---|
| 0 | 0.108±0.03$^{d}$ | 0.02±0.01$^{c}$ | 1.69±0.21$^{c}$ | 0.41±0.08c | 56.45±2.45$^{b}$ | 8.33±0.45$^{b}$ | 43±5.56$^{a}$ |
| 2 | 0.127±0.03$^{d}$ | 0.03±0.01$^{c}$ | 1.82±0.21$^{c}$ | 0.54±0.09c | 63.7±4.7$^{b}$ | 9.33±0.27$^{a}$ | 30.66±4.93$^{bc}$ |
| 4 | 0.242±0.02$^{a}$ | 0.07±0.01$^{a}$ | 3.01±0.14$^{a}$ | 1.10±0.11a | 84.35±4.3$^{a}$ | 10.33±0.53$^{a}$ | 33±2.64$^{b}$ |
| 6 | 0.19±0.02$^{b}$ | 0.06±0.01$^{b}$ | 2.71±0.24$^{b}$ | 0.97±0.10b | 66.69±3.2$^{b}$ | 9.83±0.28$^{a}$ | 28±2.64$^{c}$ |
| 8 | 0.163±0.04$^{c}$ | 0.04±0.01$^{c}$ | 2.08±0.12$^{c}$ | 0.51±0.09c | 69.6±5.2$^{b}$ | 8.33±0.67$^{b}$ | 35±2.66$^{b}$ |

compared to the control. The root and shoot length showed changes differently in response to $SiO_2$ NPs treatments (Table 1). The shoot length of the seedlings at 2, 4, and 6 g $l^{-1}$ of $SiO_2$ NPs were significantly increased by 1.12-, 1.24-, and 1.18-fold, respectively, as compared with the control. But, root length declined significantly at different concentrations of $SiO_2$ NPs and these values at 2, 4, 6, and 8 g $l^{-1}$ of $SiO_2$ NPs were 28.70%, 23.26%, 34.88%, and 18.60% lower than the control, respectively.

The RWC in *A. gilanica* subjected to different concentrations of $SiO_2$ NPs were compared with the control (Table 1). The highest RWC in *A. gilanica* were observed at 4 g $L^{-1}$ of $SiO_2$ NPs with a 49.42% increase as compared to control.

A cross-section of *A. gilanica* shoot under different concentrations of $SiO_2$ NPs (0, 2, 4, 6 and 8 g $L^{-1}$) was presented in Fig 1. Stele diameter, xylem vessels, phloem area and metaxylem number in *A. gilanica* shoot increased significantly at 2, 4, and 6 g $L^{-1}$ of $SiO_2$ NPs, but reduced at 8 g $L^{-1}$ as compared to control (Fig.2). $SiO_2$ NPs also increased metaxylem size at 4, 6, and 8 g $L^{-1}$ as compared to control. Xylem number showed a 48.5%, 45.4% and 33.08 % increase at 2, 4 and 6 g $L^{-1}$ of $SiO_2$ NPs, respectively. The increase of xylem number was related to *A. gilanica* growth parameters (Fig.2).

Total phenol contents in treated and untreated *A. gilanica* seedlings are presented in Fig.3. The obtained results showed that total phenol contents in all treatments, including 2, 4, 6, and 8 g $L^{-1}$ of $SiO_2$ NPs were elevated by 1.29-, 1.30-

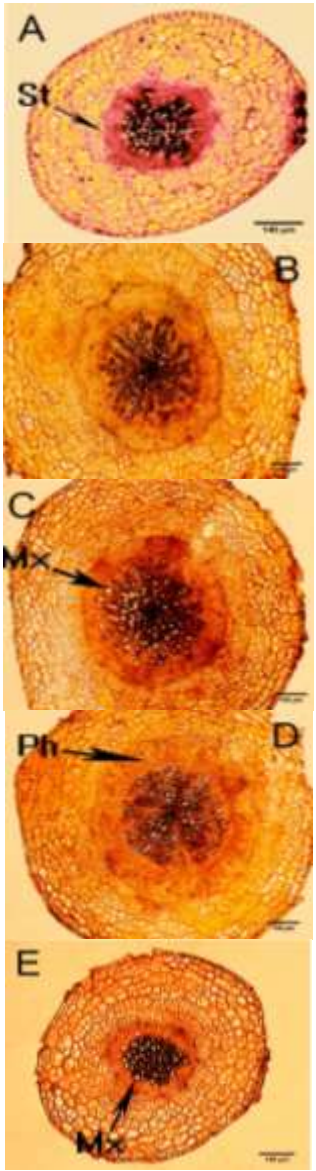


Fig. I. Cross-section *A. gilanica* shoots (2 cm over the root) under different concentrations of $SiO_2$ NPs (0, 2, 4, 6, and 8 g $l^{-1}$); St: Stele, Mx: Metaxylem, Ph: phloem

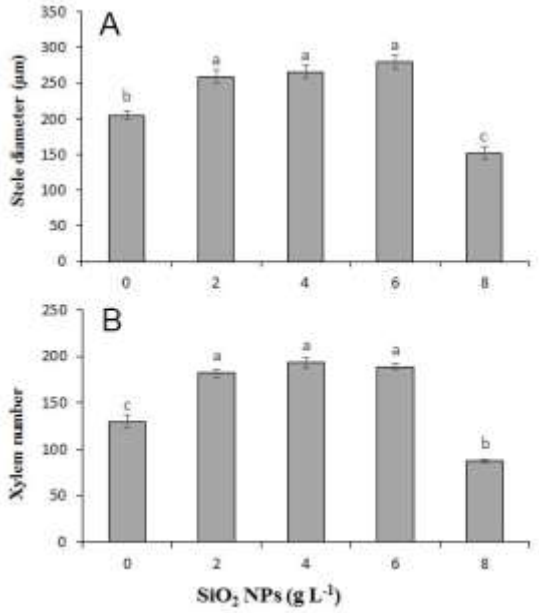


Fig. II. Changes of stele diameter (A) and xylem number (B) in *A. gilanica* seedlings under different concentrations of $SiO_2$ NPs (0, 2, 4, 6, and 8 g $l^{-1}$); means with different letters indicate a significant difference at $p \leq 0.05$ according to one-way ANOVA. Values are a mean of four experiments ± SE.

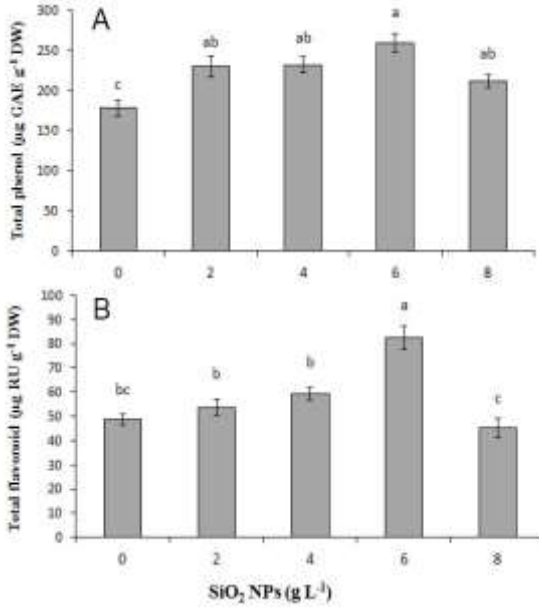


Fig. III. ChanIes of total phenol (A) and total flavonoid (B) in A. gilanica seedlings under different concentrations of $SiO_2$ NPs (0, 2, 4, 6, and 8 g $l^{-1}$); means with different letters indicate a significant difference at $p \leq 0.05$ according to one-way ANOVA. Values are a mean of four experiments ± SE.

, 1.46-, and 1.19-fold, respectively, compared to the control condition. The contents of total flavonoid in *A. gilanica* seedlings that were treated with 2, 4, and 6 g $L^{-1}$ of $SiO_2$ NPs were 1.10-, 1.21-, and 1.69-fold greater than that of the control seedlings, respectively. These results indicated that the application of $SiO_2$ NPs had a positive impact on the total flavonoid up to 6 g $l^{-1}$; however, this positive impact decreased at higher concentrations (8 g $l^{-1}$ of the NPs).

The antioxidant activity of root and leaf extracts of *A. gilanica* was determined by DPPH free radical scavenging assay. DPPH is characterized as a relatively stable free radical compound that is able to readily accept an electron or hydrogen atom to form a stable diamagnetic molecule and has been used to measure free radical scavenging activity (Nimse and Pal, 2015). The lower $IC_{50}$ value indicates the higher DPPH radical-scavenging power while the bigger $IC_{50}$ value indicates the smaller antioxidant activity. As shown in Fig. IV, the $IC_{50}$ values in leaf and root extracts of *A. gilanica* seedlings declined along with the increase in $SiO_2$ NPs concentrations. The leaf extract with the $IC_{50}$ value of 4.22 µg $ml^{-1}$ at 6 g $L^{-1}$ of $SiO_2$ NPs showed the

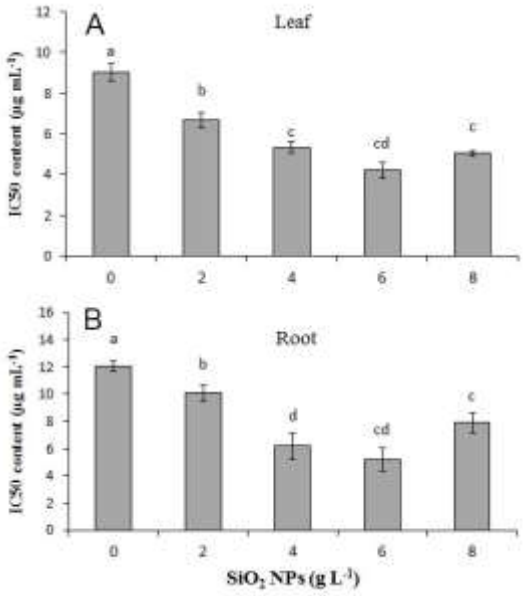


Fig. IV. Effect of different concentrations of $SiO_2$ NPs (0, 2, 4, 6, and 8 g $l^{-1}$) on $IC_{50}$ activity of leaf (A) and root (B) extracts in *A. gilanica* seedlings; bars indicate ±SE (n = 4) in each group. Different letters indicate significant differences at $p \leq 0.05$.

greatest DPPH free radical quenching activity in comparison with the other treatments (Fig. IV). Also, our results revealed that the root extract is a free radical scavenger. Increasing $SiO_2$ NPs

treatment significantly decreased the $IC_{50}$ value of the root extract compared to the control (Fig. IV).

## Discussion

In this study, growth parameters were investigated by measuring plant fresh and dry weights, root and shoot lengths, and RWC in *A. gilanica* seedlings under different concentrations of $SiO_2$ NPs.

The application of different concentrations of $SiO_2$ NPs caused an increase in the root and shoot dry and fresh weights of *A. gilanica* seedlings compared to control plants (Table 1), and seedlings treated with 4 g $l^{-1}$ of $SiO_2$ NPs had the greatest root and shoot dry and fresh weights compared to their respective controls. Improvement of root and shoot growth in the presence of Si NPs has been previously reported in *Lycopersicum esculentum* (Haghighi et al., 2012), *Lens culinaris* (Sabaghnia and Janmohammadi, 2015), *Crataegus aronia* (Ashkavand et al., 2015), Tall wheatgrass (Azimi et al., 2014), and *Zea mays* (Suriyaprabha et al., 2013b). Increased growth under $SiO_2$ NPs may be attributed to high levels of photosynthetic and transpiration rate (Xie et al., 2012), chlorophyll parameters (Xie et al., 2012), content of proteins, total free amino acids, contents of nitrogen (N), phosphorus (P), potassium (K), activities of POD and SOD (Li et al., 2012), and other biochemical processes that positively affect plant growth. Moreover, water uptake and its transportation are facilitated in the plants under NPs (Karimi and Mohsenzadeh, 2016). It seems that increased growth at 4 g $l^{-1}$ $SiO_2$ NPs may be related to more water uptake by vessels tissues, and probably $SiO_2$ NPs effect on the vessels' number and size in *A. gilanica*.

In this research, the gradual increase of the shoot length was in response to increased $SiO_2$ NPs concentrations from 0 to 4 g $l^{-1}$. At 6 g $l^{-1}$, the parameter decreased but was still higher than the control; at 8 g $l^{-1}$, the response returned to the control values. Consistent with the obtained results, the elevated shoot length has been recently reported in *Triticum aestivum* (Mushtaq et al., 2017) and *Thymus kotschyanus* under different concentrations of $SiO_2$ NPs (Abbasi Khalaki et al., 2016). The degree of increase in shoot length appeared to depend mainly on the concentration of NPs used (Thakur et al., 2018). The increase in shoot length of seedlings in the presence of NPs might be due to the nutritional behavior of particles or of dissociated ions but at nonlethal concentration (Zafar et al., 2016). Root length in *A. gilanica* seedlings significantly declined in response to increased $SiO_2$ NPs as compared to the control. Similar to our findings, a decrease of root elongation under different concentrations of alumina NPs was previously reported in the plant species corn, cucumber, soybean, cabbage, and carrot (Yang and Watts, 2005).

RWC is a physiological parameter to assess the water status of plants affected by irrigation regimes (Ashkavand et al., 2015). Our result revealed that RWC in *A. gilanica* seedlings was significantly elevated by 49.42% at 4 g $l^{-1}$ of $SiO_2$ NPs as compared to the control. This result is in contrast to the recent findings for hawthorn seedlings under different concentrations of $SiO_2$ NPs (Ashkavand et al., 2015). They showed that RWC was not affected by the $SiO_2$ NP pre-treatments. However, a positive relationship was observed between water uptake and growth parameters in *A. gilanica* leaves.

Anatomical alteration in plants is an adaptation mechanism to alleviate unfavorable environmental conditions. Vessel development can contribute to more convenient water and sap flow in the xylem and the translocation of photo assimilation in phloem (Kozlowski, 1997), which then affects the plant growth. A cross-section of *A. gilanica* shoot revealed that $SiO_2$ NPs at proper concentrations can induce metaxylem size and number. Yuan et al. (2018) found that NPs promoted the development of vascular bundles in *Capsicum annuum*. It was found that $SiO_2$ NPs with the development of vessels tissues stimulated water transport and sap flow and helped growth induction in *A. gilanica.*

Phenolic compounds are a major group of secondary metabolites that act as ROS scavenging agents and their synthesis is triggered in response to environmental stresses (Cheynier et al., 2013; D'Souza and Devaraj, 2010). In our study, the treatments caused a significant increase in both total phenolic and flavonoid contents as compared with those of the control. The greatest amounts of total phenolic and flavonoid contents were found

at 6 g $l^{-1}$ of $SiO_2$ NPs. Increased phenolic compounds could be a key mechanism in $SiO_2$-treated *A. gilanica* to scavenge ROS in cells. It is widely known that plant phenolic compounds such as flavonoids are powerful antioxidants and may act as reducing agents, singlet oxygen quenchers, hydrogen atom donators, and metal chelators. Therefore, these bioactive compounds may assist in the detoxification routes of free-radicals generated by oxidative stresses (Asif, 2012; Huda-Faujan et al., 2009). There is some evidence of the role of phenolic and flavonoid compounds in plant adaptation to abiotic stresses such as silica NPs (Miao et al., 2010). Induction of phenolic and flavonoid biosynthesis has been recently reported in *Cotton* Plant (Shallan et al., 2016), *Musa* sp. (EL-Kady et al., 2017), and rice (Suriyaprabha et al., 2012) under NPs treatments. The reason may be eliciting pathways of polyphenols biosynthesis or enhancing enzymatic activity like polyphenol oxidase activity enforced by stress conditions (Di Ferdinando et al., 2012). Polyphenol oxidase catalyzed the oxidation of various phenolic compounds to quinones using oxygen as an electron acceptor (Zhou et al., 2017).

$IC_{50}$ content significantly decreased as compared to control. The decreased $IC_{50}$ value in the seedlings treated with $SiO_2$ NPs may be attributed to higher radical-scavenging activity (Lidon et al., 2004). Increased antioxidant activity may be due to more accumulation of phenolic and flavonoid compounds in the plants treated with 6 g $l^{-1}$ $SiO_2$ NPs. Therefore, this was considered as the best concentration for the production of valuable secondary metabolites.

## Conclusion

The present study investigated the effect of $SiO_2$ NPs on *A. gilanica* seedlings. Different concentrations of $SiO_2$ NPs affected the growth, RWC, and phenolic and flavonoid contents in a concentration-dependent manner. Among various concentrations used in the study, 4 g $l^{-1}$ of $SiO_2$ NPs was the most effective treatment for the improved growth parameters of *A. gilanica* seedlings whereas it had weaker positive impacts on these parameters at higher levels (6 g $l^{-1}$). Also, phenolic and flavonoid contents elevated at 6 g $l^{-1}$ $SiO_2$ NPs, thus this concentration has a positive effect on the content and chemical composition of valuable secondary metabolites. Considering the economic significance of the medicinal plant of *A. gilanica*, these results can be used in diverse industrial sectors, such as foods, cosmetics, and pharmaceuticals.

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