Nanoparticles induced antioxidative compounds in *Matricaria chamomilla*

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Abstract

In this study, the effects of different concentrations of silica nanoparticles (NPs) were studied on growth, membrane stability, and antioxidant properties of *Matricaria chamomilla in vitro*. The sterilized seeds were incubated in different concentrations of silica NPs (0, 2, 4, 6 g L^{-1}) for one hour and then were cultured on Murashing and Skoog medium. Silica NPs application enhanced relative water content and fresh and dry weight of leaf and root. The highest growth was observed at 4 g L^{-1} silica NPs. Hydrogen peroxide and malondialdehyde significantly reduced at 4 g L^{-1} silica NPs. Total phenol and flavonoid contents increased by silica NPs treatment, and induction effect of silica NPs was more prominent at 6 g L^{-1} silica NPs. Low level of IC50 was detected at 6 g L^{-1} silica NPs. Overall, application of silica NPs at proper concentration can improve growth and induces the production of metabolites in *M. chamomilla*.

Keywords: flavonoid; hydrogen peroxide; *Matricaria chamomilla*; phenol; silica nanoparticle

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Introduction

Matricaria chamomilla is an annual plant of the Asteraceae family with thin spindle-shaped roots only penetrating flatly into the soil. The branched stem is erect, heavily ramified, and grows to a height of 10-80 cm. *M. chamomilla* has the highest anti-inflammatory effect among medicinal herbs, and has been used in herbal remedies for thousands of years. Flowers of this plant are applied in industry and medicine. This plant has many applications as anti-fungal, antibacterial, anti-allergic, anti-antispasmodic, and analgesic drug. *M. chamomilla* is rich in flavonoids including apigenin, apigenin 7-glucoside that are effective antioxidants in neutralizing free radicals (McKay and Blumberg, 2006; Singh et al., 2011).

Antioxidants are important defensive factors against free radicals. Antioxidants exert their physiologic roles by free radical detoxification. Flavonoids and the other phenolic compounds are known as plant secondary metabolites that hold an aromatic ring bearing at least one hydroxyl groups. More than 8000 phenolic compounds as naturally occurring substances from plants have been indicated. These compounds are known as the largest

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phytochemical molecules with antioxidant properties from plants (Kukic et al., 2006; Kumar and Pandey, 2013).

There is a great demand to *M. chamomilla* in the world market due to its extensive medicinal values and impeccable pharmacological properties. Also, there has been a rise in the use of natural substances instead of synthetic chemicals because many herbal medicines are free from side effects, easy to obtain, and healthy.

Nanoparticles (NPs) are unit materials with a characteristic dimension from 1 to 100 nm and remarkable structural and physicochemical characteristics. Recently, production of the crops using macronutrients and micronutrients in the form of NPs is significantly increased in agriculture. NPs cause many morphological and physiological changes in plants. Lin and Xing (2008) and Lin et al. (2009) demonstrated that NPs can enter into plant roots *via* the apoplastic pathway and they are transported to shoots through the vascular system. Thus, their uptake depends on chemical composition, size, surface coating, reactivity, concentration, and plant species (Bakshi et al., 2015; Monica and Cremonini, 2009). Silica (SiO_2) NPs are metal oxide particles which can affect plant growth, nutrient contents, and antioxidant enzymes activities (Siddiqui and Al-Whaibi, 2014; Haghighi et al., 2012; Li et al., 2012). There is little information about the effect of silica NPs on antioxidative activity and metabolites in plants and until now the *in vitro M. chamomilla* plants have not been investigated. So, the purpose of the current study was to assess the impact of different concentrations of silica NPs on growth, antioxidant properties, phenolic compounds, flavonoids, and membrane stability of *in vitro M. chamomilla* Plants.

Material and Method

Plant material and culture conditions

Seeds of *M. chamomilla* were surface sterilized in 10% (v/v) sodium hypochlorite solution for 15 min, followed by three washes with sterile distilled water. The sterilized seeds were placed in 10-cm Petri dishes containing different concentrations of silica NPs (0, 2, 4, 6 g L^{-1}) for 1 hour and then were cultured on Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962) at a temperature of 25 °C, relative humidity of 55%, and a 16-h photoperiod with a light intensity of 2300 Lux. Each treatment was carried out in triplicate and seedlings were collected after 4 weeks and kept at −70 °C and used for all the experiments.

Growth parameters

Fresh and dry weights were measured as the growth parameters. Relative water content (RWC) of leaves was estimated according to Wheatherley (1973) and based on the following equation:

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

Saturated weight (SW) of the plants was determined by keeping them in de-ionized water at 4 °C in the dark for 24 h, and DW was obtained after oven drying at 45 °C for 72 h.

H_2O_2 content and lipid peroxidation

Hydrogen peroxide (H_2O_2) content was estimated via Velikova et al. (2000) method. Leaf tissue (1 g) was homogenized in 0.1% TCA before it was centrifuged at 12000 rpm for 15 min. Supernatant (0.5 ml) was added to 0.5 ml potassium phosphate buffer (pH 7.0) and 1 ml potassium iodide (1 M) and the absorbance was recorded at 390 nm.

Malondialdehyde (MDA) content was determined through Heath and Packer (1968) procedure. Leaf tissue (1 g) was homogenized in 0.1% TCA and then was centrifuged at 13000 rpm for 10 min. The supernatant (0.5 ml) was mixed with 1ml of thiobarbituric acid (0.5%) in 20% TCA. The mixture was heated in 95 °C for half an hour and then was centrifuged at 13000 rpm for 15 min. The absorbance of the supernatant was recorded at 532 and 600 nm.

Total phenol and flavonoid content

In order to prepare methanolic extract, 2 g dry tissue was homogenized in 5 ml methanol 80% and then it was centrifuged at 5000 rpm for

20 minutes. For the total phenol content measurement, 0.1 ml methanolic extract was mixed with 2.5 ml Folin–Ciocalteu reagent 10%. The mixtures were neutralized by sodium bicarbonate 7% and then the absorbance was recorded at 765 nm (Conde et al., 1995).

Approximately 2 g dry material was homogenized in 3 ml methanol. Flavonoid content was measured using aluminum chloride colorimetric method. Methanol extract (0.5 ml) was mixed with 1.5 ml pure methanol, 0.1 ml aluminum chloride 10 %, 0.1 ml 1 M potassium acetate, and 2.8 ml distilled water and the mixture was kept at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm and was expressed in µg g^{-1} fresh weight (Chang et al., 2002).

Measurement of DPPH-radical scavenging activity

For determination of DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity Abe et al. (1998) method was used. Dry materials (100 mg) was homogenized in 1 ml ethanol 96% and then insoluble materials were removed by centrifuging at 3500 × g for 5 min. Twenty (20) µl of the extracting solution was mixed with 800 µl DPPH (0.5 mM in ethanol). The absorbance of the resulting solution was measured at 517 nm after 30 min in darkness. The antiradical capacity was expressed as IC50 (µmol ml^{-1}). The inhibitory concentration (IC50) of the seedlings needed to inhibit 50% of the DPPH radicals obtained from the standard curve was compared to that of the standard antioxidants (vitamin C (50-300 mM)). The ability to scavenge the DPPH radical was calculated as below:

Inhibition of DPPH radical (%) = [(Absorbance of control - Absorbance of sample) / (Absorbance of control)] × 100

Statistical Analyses

Each data point was the average of three replicates. The obtained data were analyzed by analysis of variance using SPSS (version 21). The significance of differences was determined according to Duncan's multiple range tests at 0.05 level of probability.

Fig. I. Effects of various concentrations of SiO_2 nanoparticle on growth of *M. chamomilla*.

Results

The results of measured growth parameters are shown in Figs. I and II. The study showed significant effects of silica NPs on fresh and dry weights. Silica NPs treatment enhanced fresh and dry weights of root and leaf as compared to control. The fresh and dry weights of root and leaf showed significant differences in various concentrations of silica NPs. Maximum increase in fresh and dry weights of root and leaf was recorded with 4 g L^{-1} compared to the control.

Silica NPs application had significant effect on RWC at 2 and 4 g L^{-1} where the inductions of RWC were 179.13% and 326.10%, respectively, as compared to the control (Fig. III. a).

H_2O_2 content reduced at 2 and 4 g L^{-1}as compared to control and wild type. Silica NPs treatment caused 2-fold decrease in H_2O_2 content at 4 g L^{-1}. H_2O_2 content in wild type was more

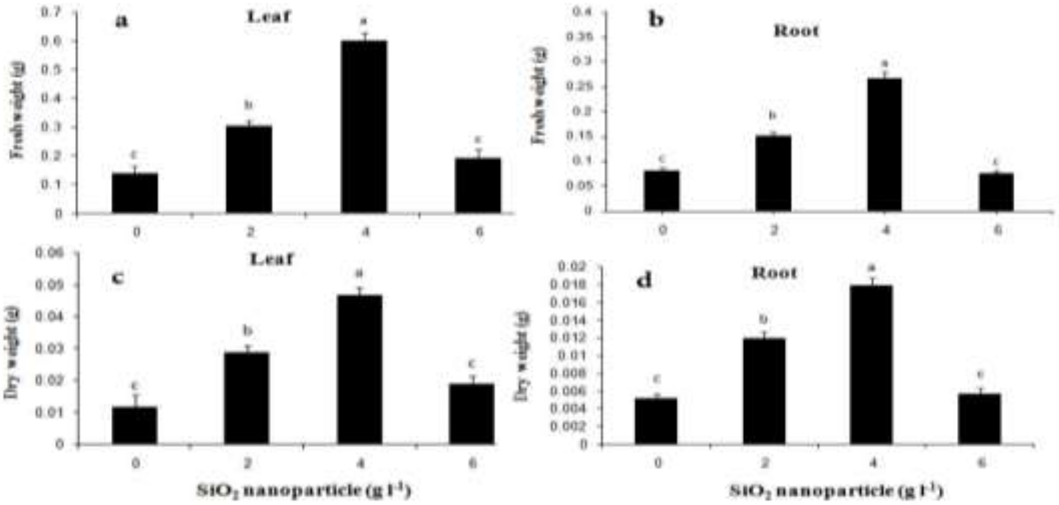

Fig. II. The effect of SiO_2 nanoparticles on fresh weight (a, b) and dry weight (c, d) of leaf and root.; vertical bars indicate means ± SE of three replicates. Different letters indicate significant differences at $P < 0.05$.

(22.26% and 51.81%) than that under 0 and 4 g L^{-1} concentrations of silica NPs (Fig. III. b).

MDA content was not significantly changed at 2 and 6 g L^{-1}, whereas this parameter declined at 4 g L^{-1}. The MDA content in 4 g L^{-1} showed 51% reduction as compared with no silica nanoparticle treatment. MDA content was much higher in wild type (2-fold) than 4 g L^{-1}silica nanoparticle (Fig. III. d).

Total phenol content was enhanced by silica NPs treatment, and the maximum increase (150.56%) was recorded at 6 g L^{-1} compared with the control. At concentrations of 4 and 6 g L^{-1}, the induction of total phenol content was 2.56 and 4.40 fold as compared to the wild type, respectively (Fig. IV. a).

Silica NPs application had significant effect on flavonoid content. Flavonoid content decreased at 2 g L^{-1} and then enhanced at 4 and 6 g L^{-1}. The maximum rise (74%) showed at 6 g L^{-1} relative to control. Flavonoid content at 6 g L^{-1} concentration was significantly higher (10.58-fold) than that of the wild type (Fig. IV. b).

The quality of the antioxidants was determined by the IC50 values with respect to ascorbic acid as standard. Increasing silica NPs treatment significantly decreased IC50 compared to the control. The maximum level of decreasing in IC50 was slightly higher at 6 g L^{-1} than other concentrations. IC50 content was more in the wild type as compared with 6 g L^{-1}concentration (Fig. V).

Discussion

Medicinal plants are valuable natural resources and are considered as the raw materials for producing harmless medicines for human. *M. chamomilla* is one of the most valuable medicinal plants which uses widely in pharmaceutical, cosmetic, and food industries. In our study, growth parameters were strongly enhanced under various concentrations of silica NPs. The highest induction of growth was observed at 4 g L^{-1}. Silica NPs application enhanced growth by an increase in non-enzymatic antioxidants. Our study suggested that silica NPs can be used for growth induction in *M. chamomilla*. Researchers have argued that silica NPs could be used as mineral fertilizers in agriculture to produce more plant crops. Siddiqui et al. (2014) showed that silica NPs enhanced fresh and dry weights in *Lycopersicum esculentum*. Silica NPs increased growth in *Zea mays* (Suriyaprabha et al., 2012). Enhancement of growth may relate to positive effects of silica NPs on the induction of chlorophyll synthesis and uptake of water and nutrients (Zheng et al., 2005; Bao-shan et al., 2004).

Environmental stresses increase production of reactive oxygen species (ROS) and cause oxidation of cellular components and alternation in redox status. MDA content, a product of lipid peroxidation, has been considered as an indicator of oxidative damage (Shalata et al., 2001). Based on our finding, H_2O_2 and MDA content showed 65% and 51% decrease at 4 g L^{-1} concentration as compared to the control, respectively. Therefore, due to the low level of H_2O_2 and MDA, the damage to the cell was lower at this concentration, so this concentration is suggested for optimal growth of *M. chamomilla*. Karimi and Mohsenzadeh (2016) showed SiO_2 NPs at high concentrations (such as 400 and 800 mg/l) have adverse effects on wheat seedlings and MDA content significantly increased, but 50 and 100 mg L^{-1} did not affect MDA content. SiO_2 application led to the decline in the electrolyte leakage (Karmollachaab et al., 2013) and MDA content (Pei et al., 2010) of plants under water stress conditions. It was found that growth induction in *M. chamomilla* may relate to more ROS scavenging under proper concentration of silica NPs and the decline in oxidative damage.

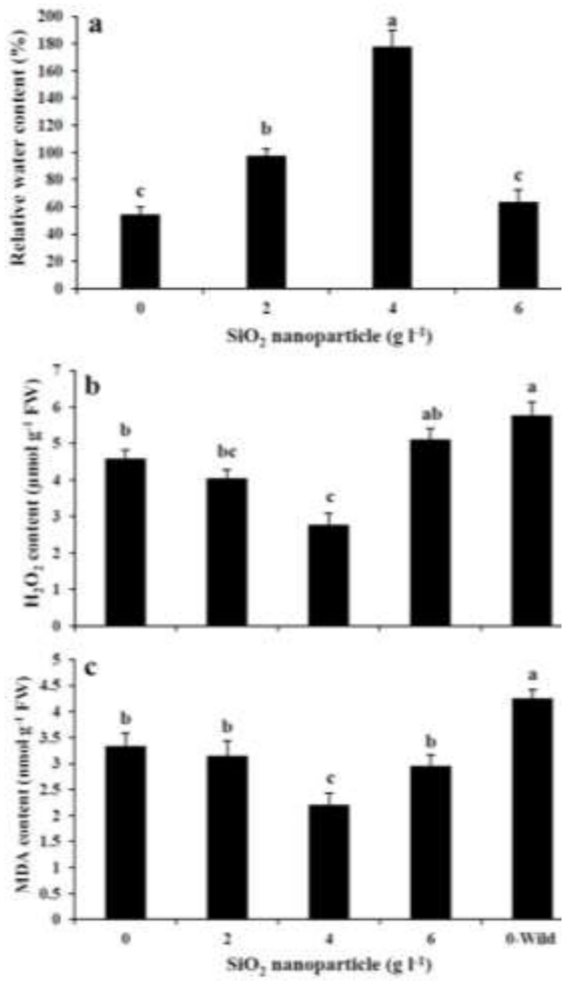

Fig. III. Changes in RWC (a), H_2O_2 content, and MDA content in *M. chamomilla* subjected to various SiO_2 nanoparticle concentrations; vertical bars indicate means ± SE of three replicates. Different letters indicate significant differences at P<0.05.

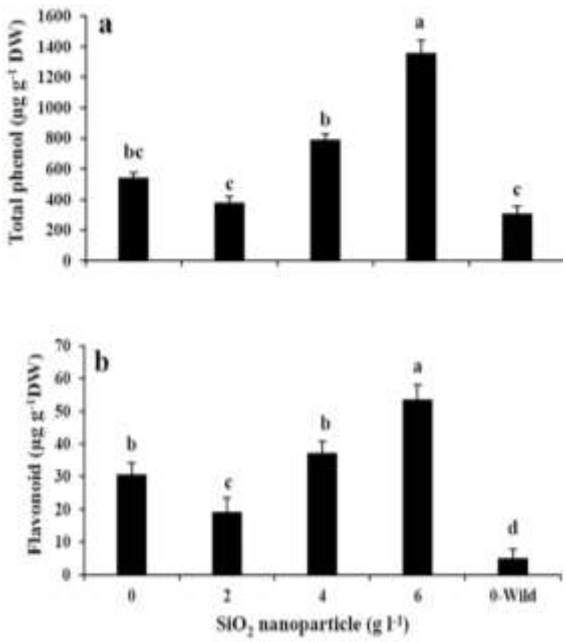

Fig. IV. The effect of SiO_2 nanoparticles on total phenol (a) and flavonoid (b); vertical bars indicate means ± SE of three replicates. Different letters indicate significant differences at P < 0.05.

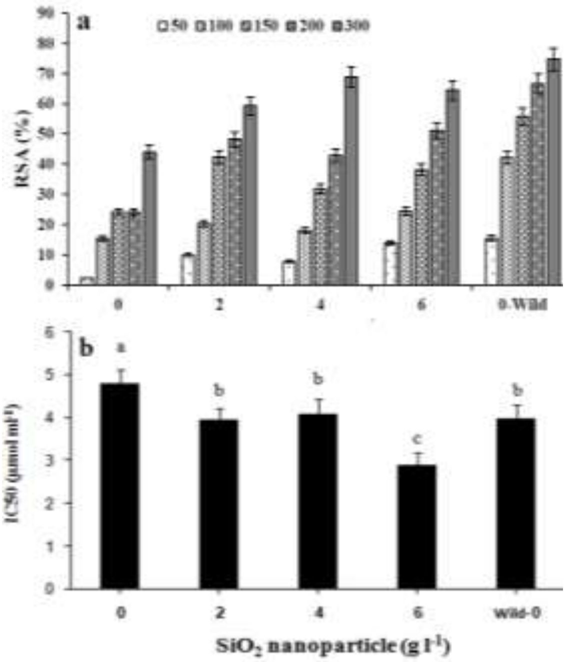

Fig. V. RSA (a) and IC50 (b) content in *M. chamomilla* under different concentrations of SiO_2 nanoparticle.

There are many reports on biological and pharmaceutical properties of phenolic compounds including antioxidant, anti-cancer, anti-bacterial, antiviral and anti-inflammatory properties (Báidez et al., 2007). Antioxidant metabolites such as phenolic compounds and flavonoids play an important role in reducing the negative effects of ROS. Phenolic compounds are synthesized from the shikimic acid pathway (Ma et al., 2014). Silica NPs caused stress tolerance in soybean by increase metabolites such as phenols (Miao et al., 2010). In the present study, it was observed that the content of phenols and flavonoids increased by increasing the concentration of NPs. At 6 g L^{-1}, the induction of phenol and flavonoid content were 2.5 and 1.74 fold as compared to the control, respectively. IC50 content significantly decreased as compared to the control. Decrease in IC_{50} value may be directly related to a higher radical-scavenging activity. Therefore, this concentration was considered as a suitable treatment for the production of useful metabolites due to increase in phenol and flavonoid content, and reduction of IC50.

In conclusion, based on the significant growth of *M. chamomilla* and low content of H_2O_2 and MDA at 4 g L^{-1} silica NPs, this treatment can be considered as the best condition for this plant. Contents of secondary metabolites enhanced at 6 g L^{-1} silica NPs; thus, this treatment was suitable for the production of valuable metabolites.

The essential oils of *M. chamomilla* contain significant bactericidal and antifungal compounds. Considering the importance of this plant as a source for a large number of pharmaceutical substances, further studies are proposed on the field of medicine.

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