



Combined foliar and soil application of silica nanoparticles enhances the growth, flowering period and flower characteristics of marigold (*Tagetes erecta* L.)

Eman A. Attia^a, Nevien Elhawaw^{b,c,*}

^a Ornamental Plants and Landscape Gardening Research Department, Horticultural Research Institute, Agricultural Research Center, 12619 Giza, Egypt

^b Department of Agricultural Botany, Plant Physiology and Biotechnology (MEK), Debrecen University, Böszörményi Street 138, 4032 Debrecen, Hungary

^c Department of Biological and Environmental Sciences, Faculty of Home Economic, Al-Azhar University, Tanta 31732, Egypt

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ABSTRACT

In recent years, the utilization of nanoparticles, especially in agricultural sector, has been intensively gained the interest of policymakers and the public. Most of these nanoparticles are applied singularly through foliar or soil applications. However, no reports are highlighting the interaction between foliar and soil application of nanoparticles, particularly silica nanoparticles (SiNPs). This study reports the differences between the singular and combined (foliar and soil) applications of SiNPs on the ornamental model marigold (*Tagetes erecta* L.) using different concentrations of amorphous SiNPs in pot experiments carried out under open field conditions during two consecutive seasons (2017 and 2018). The results proved the potentiality of SiNPs on plant growth where all the treated plants, regardless of the SiNPs concentration and delivery technique, displayed higher values for all the measured parameters compared to the control plants. Moreover, results demonstrated that the soil application of 200 mg L⁻¹ resulted in higher values of plant height, number of branches, and root length than the foliar application of the same SiNPs concentration. On the other hand, the foliar application of 200 mg L⁻¹ significantly increased flower's traits over the soil application. The enhancement effect of SiNPs on plant biometrics, physiology, and properties of flower strongly linked to the Si content in plant leaves. The highest Si content corresponded to the combined foliar and soil application. Consequently, the higher number of flowers, flower's diameter, fresh and dry masses of flower, flowering period, and days taken to first bud initiation linked to plants received jointly 200 and 600 mg L⁻¹ through foliar and soil application, respectively.

1. Introduction

Silicon (Si) is the second most abundant element in the Earth's crust following oxygen. The essentiality of Si to higher plants particularly dicots is not proved yet; however, plant physiologists and agronomists classify Si as a benefit element to plants (El-Ramady et al., 2018). Si benefits plants by enhancing their tolerance capacity to biotic and abiotic stress such as pests, fungal contravention, salinity, and drought (Alsaeedi et al., 2017a, 2018; 2019a,b). Also, Si helps plants to resist the toxicity of several toxic metals such as aluminum, copper, manganese, zinc, and iron (Ma, 2004). Si protects plants against drought and wilting by increasing the water-use efficiency and reducing water losses via evapotranspiration (Alsaeedi et al., 2019a,b). In the past, there was no interest in adding Si to the plant due to the abundance of Si in the soil.

Recently, with the increase in agricultural intensification, there is a need for adding Si to improve plant growth and increase its ability to resist both biotic and abiotic stresses (El-Ramady et al., 2018). There are different forms of Si are used as fertilizers such as silicic acid, amorphous silica, and organic complexes; however, the phytoavailability of most of them is low. The most common and available sources of Si that plants generally absorb are monosilicic and polysilicic acids. Taken Si by plant roots undergoes polymerization in root tissues then transported and accumulated in stem and leaf tissues. The deposition of Si in the aboveground part is attributed to the transpiration and changed later into amorphous silica in the cell wall of vascular tissues leading to a reduction in water loss (Ma and Yamaji, 2006). Phytoavailability of Si depends on several factors, i.e., Si concentration in soil solution, organic matter, soil pH, and soil moisture content (Alsaeedi et al., 2019a,b).

* Corresponding author at: University of Debrecen, Böszörményi ut 138, 4032, Debrecen, Hungary.

E-mail addresses: nader.1234225@gmail.com (E.A. Attia), nevienadelismailhawaw@azhar.edu.eg, nevienelhawaw@gmail.com (N. Elhawaw).

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Plants take up Si via two proposed mechanisms, i.e., active and passive transportation, depending on plant species. Several plant species are known for their passive uptake of Si such as grasses. On the other hand, a gene embedded in the plasma membrane of exodermis and endodermis layers leads to an excess Si accumulation in xylem via active pathway transportation as previously reported in rice (Ma et al., 2006).

In recent times, interest in several substances in their nanoscale (nanoparticles) has increased, as it has begun to be utilized in various fields, including medicine, industry, electronics, wastewater treatment, and agriculture (Elhawat et al., 2018). In the agricultural sector, many nanoparticles have been used to increase plant resistance to different biotic and abiotic stresses as well as enhance its productivity. Recently, the nanoscale silica, as a main source of Si, has gained an extraordinary in agricultural production due to its large surface area and tiny size, which guarantees feasible penetration of SiNPs into root cells. As a consequence of these unique characters, SiNPs are potentially introduced into agriculture particularly to mitigate the abiotic stresses (Alsaedi et al., 2019a,b). Several studies reported that SiNPs enhance plant growth, its physiology and morphology through different ways such as adding a structural color to the plants by forming a binary film at the cell wall of the epidermis (Strout et al., 2013; Suriyaprabha et al., 2014). Additionally, SiNPs strengthen the cell wall preventing fungal, bacterial, and nematodal infections, and thus support plant growth under biotic stress and increasing the yield. Also, SiNPs were reported to decrease evapotranspiration by reducing stomatal conductance (Rastogi et al., 2019). Sadgrove (2006) reported that SiNPs play an important role in improving some soil properties such as water retention, soil texture, soil erosion, cation exchange capacity, and stability of organic substances. The application of nanomaterials (nanofertilizers) to crops lacks a comprehensive investigation. Both foliar and soil applications have been used; however, no literature discussed the pros and cons of foliar application versus soil application of nanoparticles, especially SiNPs.

Marigold (*Tagetes erecta*) is an important commercial flower. The genus *Tagetes* consists of approximately 40–50 species and belongs to family Asteraceae (Composite). It is very popular due to easy to grow and wider adaptability. Its flowers are rich in lutein, therefore petals are used in pigments extraction industry specially lutein. Also flowers are used in drugs, and pharmaceutical products, processed food, confectionery and in the poultry industry; one of the most important effects of the plant is their use as very valuable intercrop for controlling plant parasitic nematodes and insecticidal activity (Darwish, 1992). Another advantage of the marigold plant is being served as a bioagent to control some diseases. For instance, powdered marigold roots were reported to control root-knot nematodes, and the extracts prepared from the fresh tissues of marigold plants were cited to suppress cabbage fly (*Delia radicum*) (Leger and Riga, 2009).

The present study aimed to investigate the effect of the application method of SiNPs on the marigold plants using foliar, soil applications and their combination. Also, the effect of SiNPs on growth, flowering and flower's characteristics of the marigold plants was evaluated.

2. Materials and methods

2.1. Experimental location and plant materials

Pot experiments under open field conditions were conducted at the experimental farm of the Horticultural Research Station, Sakha, Kafr El-Sheikh, Egypt, during two consecutive seasons of 2017 and 2018 to study the performance and development of marigold (*T. erecta*) plants treated with different concentrations of nanosilica (SiNPs) suspensions. Also, flower characteristics and biomass yield were evaluated. Marigold seeds were bought from Harraz for Food Industry & Natural Products Company, Cairo, Egypt. Seed sowing was carried out on the first of March of the two seasons in a tray (209 cells) filled with a nursery medium of peat and vermiculite at a 2:1 ratio. Seed sowing was done at

the rate of one seed per cell and tap water was applied for irrigating seedlings.

2.2. SiNPs suspensions

The SiNPs suspensions were prepared using synthesized hydrophilic silica nanoparticles (Aerosil³⁰⁰, Evonik Industries, Germany). Hydrophilic silica is a white powder of high purity amorphous silica which is 130 moistened with water and can be dispersed in water. The characteristics of the SiNPs provided by the manufacturer were: specific surface area (270–330 m² g⁻¹), mean diameter (10 nm), and pH (3.7–4.5). The SiNPs were dispersed in deionized water, using an ultrasonic water bath (Elmasonic E60H, Germany) for 2 h at 45 °C, to prepare suspensions with concentrations of 100, 200, 400, and 600 mg L⁻¹.

2.3. Experimental design and plant culture

Two-month-old uniform marigold seedlings (12–15 cm height) were transplanted in plastic pots (20 cm diameter x18 cm depth) filled with 3.8 kg air-dried growth medium (a mixture of soil: sand at 1:1 ratio) per pot at a rate of one seedling per pot. Soil characteristics are: clay 62.00 %; silt 26.70 %; sand 2.28 %; soil texture clayey; pH 7.80; electrical conductivity (EC) 0.79 dS m⁻¹; Ca²⁺ 2.32 meq L⁻¹; Mg²⁺ 1.75 meq L⁻¹; Na⁺ 4.15 meq L⁻¹; K⁺ 0.80 meq L⁻¹; HCO₃⁻ 3.20 meq L⁻¹; CO₃²⁻ not detected; Cl⁻ 4.00 meq L⁻¹ and SO₄²⁻ 1.54 meq L⁻¹. Sand characteristics are: clay 2.60 %; silt 00.00 %; sand 97.40 %; soil texture sandy; pH 7.70; EC 0.58 dS m⁻¹; Ca²⁺ 3.20 meq L⁻¹; Mg²⁺ 1.15 meq L⁻¹; Na⁺ 1.30 meq L⁻¹; K⁺ 0.15 meq L⁻¹; HCO₃⁻ 2.66 meq L⁻¹; CO₃²⁻ not detected; Cl⁻ 1.36 meq L⁻¹ and SO₄²⁻ 1.78 meq L⁻¹. The experimental pots were arranged in a Randomized Complete Block design including 12 treatments of different SiNPs suspensions (mg L⁻¹) added through foliar application (F), soil application (S) or their combination (F + S) with 9 repeats as follows: T1 (control; tap water); T2 (100 F); T3 (200 F); T4 (200S); T5 (400S); T6 (600S); T7 (100 F+200S); T8 (100 F+400S); T9 (100 F+600S); T10 (200 F+200S); T11 (200 F+400S); and T12 (200 F+600S). The foliar application of SiNPs was done over the highest point of the plant at a rate of 97.2 mL pot⁻¹, by utilizing pressurized spray bottle 1 L with 0.1 % Tween® 20 (Sigma-Aldrich, Hungary) as an adjuvant. The foliar application for the respective treatment was repeated three times during the growing season at one (8th May), three (22nd May), and five (6th June) week(s) after transplantation. Soil application of SiNPs was added to the soil one day before the transplanting of the seedlings (30th April) and four weeks later (1st June) in volume 1000 mL water per pot to stay safely below the water holding capacity of the soil (water holding capacity, WHC, which was 400 mL kg⁻¹ soil at saturation). Control plants were sprayed or watered by distilled water at the same time as foliar or soil application of SiNPs. Pots were placed under open field conditions and the soil moisture content was kept at no higher than 75 % water saturation during the entire period of the experiment (checked by weighing the pot each time it was watered). Marigold plants were fertilized with NPK at a rate of 8 g pot⁻¹ ammonium sulfate (20.5 % N), 4 g pot⁻¹ triple superphosphate (P₂O₅), and 4 g pot⁻¹ potassium sulfate (48.5 % K₂O). While P fertilizer was added one time before transplanting of the seedlings, both N and K fertilizers were divided into two equal doses added at 3 and 7 weeks after transplantation according to El-Morsy (2011).

2.4. Plant biometrics and flower characteristics

Plant height, from the soil surface to the plant top, was measured 60 days after transplantation with the onset of flowering bud. The number of new branches was counted 120 days after transplantation. At the harvest of plants, 210 days from transplantation, length, and dry masses of root systems were measured, where after removing plants from pots shoot and root parts were separated. To obtain the dry weight of root system, air-dried samples were dried in force-air oven at 65 °C for 48 h

until the constant weight. Days taken to first flower bud initiation and length of the flowering period were assessed. Also, characteristics of flower were measured 180 days after transplantation.

2.5. Chemical and biochemical traits of marigold plants

Chlorophyll content was assessed using SPAD meter (Minolta, Co., Ltd, Japan) on the 5th mature leaf; values recorded were an average of 9 readings. Activity of antioxidant enzymes, i.e., catalase (CAT) and peroxidase (POD), was estimated in the fifth fully-expanded leaf from the plant tip 60 days after transplantation. Briefly, 0.5 g fresh leaves tissue was homogenized in 3 mL TRIS buffer (50 mM, pH 7.8), containing 1 mM EDTA-Na and 7.5 % polyvinylpyrrolidone at 0–4 °C. The homogenates were centrifuged at 10,000 × g for 20 min at 4 °C. The measurements were carried out at 25 °C, using the model UV-160A spectrophotometer (Shimadzu, Japan). The activity of CAT was measured according to Aebi (1983), while POD activity was determined following the method proposed by Hammerschmidt et al. (1982). Silicon (Si) content was detected in the fifth fully expanded leaf at the end of the experiment (210 days from transplantation) according to Frantz et al. (2008). The absorbance of a developed blue color was measured between 10 and 30 min at 650 nm using the model UV-160A spectrophotometer (Shimadzu, Japan). The Si content was calculated using a standard curve prepared with known Si concentration.

2.6. Statistical analysis

Dependent variables were checked for normality and homoscedasticity and transformed as necessary. Data analysis was performed using Microsoft Excel 2003 and the SPSS 13.0 software package (SPSS Inc., Chicago, IL). The analysis of variance using two-way ANOVA was conducted between treatments and growing seasons, while one-way ANOVA was applied to evaluate the differences among treatments within the same growing season. The experimental layout was a Completely Randomized Design with nine repeats. Separation of means was performed by post hoc test (Tukey's test), and significant differences were accepted at the levels $p < 0.05$, 0.01 , and 0.001 . The data were presented as mean ± standard deviation (n = 9).

3. Results

3.1. Response of plant biometrics to SiNPs and application method

In general, plant biometrics follows a dose-response relationship across all SiNPs concentrations and application methods. Plant height increased significantly upon increasing the rate of applied SiNPs regardless of the application way. In the first growing season, foliar application of 100 mg L⁻¹ increased plant height by 11 % compared to control plants, while 200 mg L⁻¹ showed an increase of 18 %. Likewise, soil application of SiNPs at the rate of 200 mg L⁻¹ increased plant height by 19 % in comparison to control, whereas a 44 % increase was measured when plants received 600 mg L⁻¹ of SiNPs. Despite the positive effect of the singular application of SiNPs on plant height, the combined application of SiNPs (foliar and soil) resulted in the highest values of plant height (Table 1).

All treated marigold plants with SiNPs, regardless of concentration and application method, exhibited more branches than control plants in both growing seasons (Table 1). Increasing the applied concentration of SiNPs increased the number of branches in all the application methods used. However, the dual application of SiNPs in the form of foliar and soil applications resulted in the highest number of branches; for instance, the number of branches of plants in the treatment F200S600 was 20 while control plants had 6. The same trend was noticed in the second growing season with slightly higher values (Table 1).

Control plants had a root length of 18.6 cm, which is the shortest length of root among all the treatments (Table 1). Significantly, the

Table 1

Biometrics of marigold plants treated with different concentrations of silica nanoparticles (SiNPs) using different application methods during two growing seasons.

Year	Treatment	Plant height (cm)	Number of branches	Root length (cm)	Root DW (g plant ⁻¹)
2017	Control	45.5 ± 0.06k	6.0 ± 1.00 g	18.6 ± 0.31 h	2.2 ± 0.02 j
	F100	50.5 ± 0.06 j	6.7 ± 0.58fg	20.3 ± 0.25 g	5.1 ± 0.04i
	F200	53.7 ± 0.15 h	8.7 ± 0.58ef	22.3 ± 0.10f	5.9 ± 0.07 h
	S200	54.1 ± 0.10 h	10.3 ± 0.58de	22.7 ± 0.15ef	5.2 ± 0.04i
	S400	63.4 ± 0.10f	11.3 ± 0.58 cd	23.1 ± 0.06de	6.1 ± 0.03 g
	S600	65.3 ± 0.06d	11.7 ± 0.58 cd	23.7 ± 0.06c	9.3 ± 0.06d
	F100S200	53.7 ± 0.10i	12.3 ± 0.58 cd	23.4 ± 0.15 cd	5.8 ± 0.07 h
	F100S400	64.5 ± 0.06e	13.0 ± 1.00c	24.5 ± 0.10b	8.8 ± 0.06e
	F100S600	66.4 ± 0.06b	13.3 ± 0.58c	24.6 ± 0.10b	12.1 ± 0.03c
	F200S200	58.5 ± 0.06 g	15.7 ± 0.58b	23.0 ± 0.10de	6.4 ± 0.03f
	F200S400	66.0 ± 0.06c	17.0 ± 1.00b	24.5 ± 0.15b	12.6 ± 0.07b
	F200S600	67.5 ± 0.06a	20.0 ± 1.00a	26.6 ± 0.26a	13.0 ± 0.06a
	Control	37.3 ± 0.15i	7.0 ± 0.00 h	19.6 ± 0.15 g	3.0 ± 0.09 j
	F100	50.1 ± 0.01 h	7.3 ± 0.58 h	20.1 ± 0.15fg	5.7 ± 0.03 h
	F200	52.0 ± 0.06 g	9.3 ± 0.58 g	21.6 ± 0.21ef	6.1 ± 0.02 g
	S200	53.4 ± 0.10f	11.0 ± 1.00fg	22.8 ± 0.06de	5.4 ± 0.02i
	S400	60.7 ± 0.29d	11.7 ± 0.58ef	23.7 ± 0.15 cd	6.1 ± 0.03 g
	S600	62.6 ± 0.15c	12.0 ± 0.00def	23.7 ± 0.15 cd	10.7 ± 0.03d
2018	F100S200	53.1 ± 0.21f	12.3 ± 0.58def	23.8 ± 0.10 cd	6.1 ± 0.02 g
	F100S400	62.2 ± 0.44c	13.0 ± 0.00de	24.5 ± 0.10bc	9.8 ± 0.10e
	F100S600	66.6 ± 0.10a	13.7 ± 0.58d	25.7 ± 1.71ab	12.1 ± 0.04c
	F200S200	59.7 ± 0.10e	16.3 ± 0.58c	23.2 ± 0.15 cd	7.1 ± 0.02f
	F200S400	65.7 ± 0.15b	19.0 ± 1.00b	24.5 ± 0.21bc	12.4 ± 0.02b
	F200S600	66.9 ± 0.06a	21.7 ± 0.58a	26.9 ± 0.06a	13.1 ± 0.01a
	F-test (Two-ways)				
	Year	***	***	*	***
	Treatment	***	***	***	***
Year X Treatment	***	ns	**	***	

Data presented are mean ± SD (n = 9).

Means in the same column and within the same year followed by different letters are significant according to Tukey's test ($p \leq 0.05$).

application of SiNPs via either foliar or soil application increased the length of the root system of marigold plants; however, root length induced more in the presence of higher SiNPs concentrations. Moreover, the combined application of SiNPs through foliar and soil applications exhibited the most inductive effect recording the longest root length. While the treatment of S600 had a root length of = 23.7 cm, the root length of plants in the treatment of F200S600 was 26.6 cm (Table 1). Similar results were also measured in the second growing season that had slightly higher root lengths.

The synergetic effect of SiNPs on the growth of marigold plants was clearly noticed on root system development. The results of the root dry weight of marigold plants were similar to those reported for the fresh

weight of root upon treating plants with SiNPs. Regardless of the application way, increasing the dose of SiNPs increased the root dry weight as all the treated plants had a higher dry weight than control plants. The lowest applied concentration of SiNPs, i.e., 100 mg L⁻¹ added as a foliar application, had root dry weight higher by 132 % than control plants. On the other hand, higher SiNPs concentrations resulted in higher values of the root dry weight. Moreover, treated plants with combined application of SiNPs in the form of foliar and soil application led to the highest root dry weight. The treatment of F200S600 exhibited the highest root dry weight of 13.0 g plant⁻¹, which is higher than control plants by 491 % (Table 1). The dry weight of plant root in the second growing season was higher than those measured in the first season but the response of plants to SiNPs at the level of the concentration and application method was almost the same.

3.2. Physiology of marigold plants fertilized with SiNPs by different application methods

Marigold plants displayed a promising response to SiNPs added through either foliar or soil application (Table 2). The relative chlorophyll content (SPAD) in leaves of marigold plants increased upon the exogenous application of SiNPs through either foliar, soil application, or their dual application (Table 2). The control plants had the lowest SPAD content (23.6), which increased by 12.3 % when plants were sprayed with 100 mg L⁻¹. Foliar application of SiNPs was more effective than soil application concerning the SPAD value; applying SiNPs at the rate of 100 mg L⁻¹ via foliar application had the same value (26.5) recorded for plants received 200 mg L⁻¹ through soil application. There is no significant difference in SPAD values between the foliar addition of 200 mg SiNPs (27.5) and 400 mg SiNPs applied through soil application (27.2). However, the application of the higher concentration of SiNPs (600 mg L⁻¹) via soil application had a higher SPAD value (28.9). Upon the combined application of SiNPs (foliar and soil applications) higher SPAD values were measured; the highest SPAD value (31.6) corresponded to the treatment of F200S600 (Table 2). However, no significant differences were noticed between the treatment of F200S600 and F200S400, which recorded 32.5 SPAD value. The same results were reported within the second growing season with a slight insignificant increase.

Silicon content in the fifth fully expanded leaf from plant tip increased upon treating marigold plants with SiNPs through either foliar or soil application. Control plants had the lowest Si content (3.71 mg kg⁻¹) among all the treatments. Silicon content follows a dose-response relationship, i.e. as much as 5.01 mg kg⁻¹ at the treatment of F200 to as little as 4.15 mg kg⁻¹ at the treatment of F100. Marigold plant showed a greater response to the soil application of SiNPs than foliar application, where when the same concentration was applied, the accumulation of Si in leaf tissues was higher in case of soil application. For instance, plants treated with 200 mg SiNPs as the foliar application had Si content of 5.01 mg kg⁻¹, while when soil application was applied the Si content was 5.13 mg kg⁻¹ (Table 2). However, higher Si contents were measured in leaves of plants received SiNPs added as a combined foliar and soil application. The highest Si content (8.28 mg kg⁻¹) corresponded to plants treated with 200 and 600 mg L⁻¹ added as foliar and soil applications, respectively (F200S600 treatment). Plants displayed the same accumulation of Si in their leaf tissues in the second growing season as in the first season.

Contrary to all the previously mentioned characteristics, the activity of both antioxidant enzymes, i.e., CAT and POD, decreased with increasing SiNPs dose even within the same application method (Table 2). The reduction CAT activity follows a dose-response relationship, i.e., as little as 0.51 μmol H₂O₂ consumed mg⁻¹ protein min⁻¹ at the treatment of F200S600 to as much as 3.85 μmol H₂O₂ consumed mg⁻¹ protein min⁻¹ at the treatment of F100. All SiNPs treatments except F100 resulted in lower CAT activity than control plants, which had 3.19 μmol H₂O₂ consumed mg⁻¹ protein min⁻¹. Plants sprayed with 200 mg L⁻¹ of SiNPs had CAT activity of 0.78 μmol H₂O₂ consumed mg⁻¹

Table 2

Physiology of marigold plants fertilized with silica nanoparticles (SiNPs) by foliar, soil applications and their combinations during two growing seasons.

Year	Treatment	Chlorophyll (SPAD)	Silicon (mg kg ⁻¹)	Catalase (μmol H ₂ O ₂ consumed mg ⁻¹ protein min ⁻¹)	Peroxidase (U mL ⁻¹ min ⁻¹ g ⁻¹ DW)	
2017	Control	23.6 ± 1.15d	3.71 ± 0.01 j	3.19 ± 0.01b	3.50 ± 0.05f	
	F100	26.5 ± 0.99 cd	4.15 ± 0.02i	3.85 ± 0.02a	4.00 ± 0.03e	
	F200	27.5 ± 1.03bc	5.01 ± 0.02 h	0.78 ± 0.03 g	3.01 ± 0.07 h	
	S200	26.5 ± 0.61 cd	5.13 ± 0.02 g	0.91 ± 0.04f	8.06 ± 0.01a	
	S400	27.2 ± 1.54bc	5.32 ± 0.01f	0.69 ± 0.02 h	5.72 ± 0.06b	
	S600	28.6 ± 1.31abc	6.25 ± 0.05d	0.59 ± 0.03i	4.89 ± 0.07c	
	F100S200	26.8 ± 0.85 cd	5.57 ± 0.01e	1.22 ± 0.02d	4.48 ± 0.08d	
	F100S400	28.9 ± 1.01abc	6.68 ± 0.01c	1.15 ± 0.01e	4.38 ± 0.09d	
	F100S600	30.4 ± 0.58ab	7.42 ± 0.03b	1.12 ± 0.02e	2.73 ± 0.04i	
	F200S200	27.6 ± 0.58bc	6.61 ± 0.05c	1.96 ± 0.02c	3.87 ± 0.13e	
	F200S400	31.5 ± 1.20a	7.48 ± 0.02b	0.92 ± 0.01f	3.28 ± 0.01 g	
	F200S600	31.6 ± 2.02a	8.28 ± 0.00a	0.51 ± 0.01 j	1.48 ± 0.02 j	
	Control	23.3 ± 1.47e	4.57 ± 0.03i	2.41 ± 0.02b	2.65 ± 0.14 g	
	F100	26.5 ± 2.02de	4.88 ± 0.06 h	3.17 ± 0.04a	3.19 ± 0.08f	
	F200	27.6 ± 0.58 cd	5.50 ± 0.06 g	0.92 ± 0.01f	2.34 ± 0.03 g	
	2018	S200	26.5 ± 0.60de	4.91 ± 0.09 h	0.98 ± 0.03f	8.46 ± 0.39a
S400		26.9 ± 0.21cde	6.08 ± 0.09e	0.68 ± 0.01 g	4.86 ± 0.05c	
S600		28.4 ± 1.48bcd	6.37 ± 0.06d	0.61 ± 0.05 h	3.73 ± 0.06e	
F100S200		27.6 ± 1.03 cd	5.66 ± 0.03 g	2.05 ± 0.02c	6.49 ± 0.17b	
F100S400		28.8 ± 0.61bcd	5.94 ± 0.05ef	1.95 ± 0.04d	3.28 ± 0.00f	
F100S600		30.2 ± 0.60abc	7.23 ± 0.06b	0.95 ± 0.02f	2.41 ± 0.06 g	
F200S200		26.9 ± 0.56 cd	5.87 ± 0.03f	1.54 ± 0.04e	4.19 ± 0.07d	
F200S400		31.2 ± 1.80ab	6.86 ± 0.06c	0.93 ± 0.02f	2.58 ± 0.05 g	
F200S600		32.5 ± 1.77a	7.79 ± 0.08a	0.54 ± 0.01 h	1.81 ± 0.01 h	
F-test (Two-ways)						
Year		ns	ns	*	ns	
Treatment		***	***	***	***	
Year X Treatment		ns	**	***	*	

Data presented are mean ± SD (n = 9) Means in the same column and within the same year followed by different letters are significant according to Tukey's test (p ≤ 0.05).

protein min⁻¹ that is lower than that of those plants received the same SiNPs dose through soil application. However, the treatment of F200S600 exhibited the lowest CAT activity. Similar findings were obtained from the second growing season of the experiment keeping the same trend and responses.

In contrast to the CAT enzyme, most of the treated marigold plants had higher POD activity than control. However, within the one application method, POD activity declined upon increasing the dose of SiNPs. For instance, the soil application of 200 mg L⁻¹ had a POD activity of 8.06 U mL⁻¹ min⁻¹ g⁻¹ DW that reduced to 4.89 U mL⁻¹ min⁻¹ g⁻¹ DW at 600 mg L⁻¹. The highest POD activity (8.06 U mL⁻¹ min⁻¹ g⁻¹ DW)

was measured when 200 mg L⁻¹ were applied via the soil application (S200), while the lowest POD activity corresponded to the treatment of F200S600 (1.48 U mL⁻¹ min⁻¹ g⁻¹ DW). Foliar application of 200 mg L⁻¹ resulted in lower POD activity compared to the soil application of the same concentration (Table 2). The same tendency was noticed in the samples of the second growing season.

3.3. Flowering period and flower characteristics of marigold in presence of SiNPs applied by different methods

In general, the exogenous application of SiNPs, regardless of the application method, improved the characteristics of the flowering period and flower's traits of marigold plants compared to control plants. All treated marigold plants with SiNPs, regardless of the concentration and application way, needed shorter periods to first bud initiation. While control plants needed 120.7 days to first bud initiation, plants of the treatment of S200 reached the first bud initiation stage after 119 days. Within the singular foliar and soil applications and their combinations increasing SiNPs concentration significantly reduced the time needed to first bud initiation stage (Table 3). The highest reduction in days to first bud initiation corresponded to the combination of foliar and soil application using the highest SiNPs concentrations (F200S600), where plants needed only 90.7 days to first bud initiation with a reduction of almost 25 % compared to control plants. On the other hand, the application method seemed to significantly affect the flowering period properties in the form of days taken to first bud initiation, where the application of 200 mg L⁻¹ of SiNPs by foliar and soil applications resulted in 109 and 119 days taken to first bud initiation, respectively. Also, the combination of S600 and F200 had a shorter period to first bud initiation (90.7 days) compared to the combination of S600 and F100, which needed 95.7 days (Table 3). The statistical analysis showed

significant differences in days taken to first bud initiation between the growing seasons where longer periods were reported in the second season.

The positive effects of SiNPs on flowering characteristics of marigold plants extended to significantly affect the duration of the flowering stage. Untreated plants with SiNPs (control) had 55.7 days of duration of flowering as the shortest recorded period of flowering among all the treatments. The duration of flowering extended upon increasing the concentration of SiNPs within the one application method. The singular foliar application showed a better influence on the duration of flowering than the singular soil application. For instance, foliar and soil application of 200 mg L⁻¹ of SiNPs had a flowering period of 65.3 and 61.7 days, respectively. Likewise, the combination of foliar and soil applications of higher SiNPs concentrations exhibited a significantly positive effect on the duration of flowering than lower SiNPs concentrations. For example, the treatment of F100S200 had 61.3 days while F200S600 possessed 73.7 days of duration of flowering. Similar results were obtained from the second growing season with insignificant differences as the statistical analysis had been shown.

Length of flower stalk (cm) follows, also, a dose-response relationship across all SiNPs concentrations and application methods. Control plants had the shortest flower stalk (7.67 cm), whereas the lowest SiNPs concentration (100 mg L⁻¹ added as a foliar application) had longer flower stalk by 12 % (Table 3). The length of flower stalk increased, regardless of the application method, upon increasing the concentration of SiNPs. The treatment of F100 had a length of flower stalk of 8.60 cm, while F200 possessed a length of flower stalk of 8.93 cm. At the same concentration of SiNPs, the foliar application seemed to be better than the soil application. The combination of foliar and soil applications, regardless of SiNPs concentration, had a longer flower stalk than that in the case of the singular application of either foliar or soil application.

Table 3

Traits of marigold flower in presence of silica nanoparticles (SiNPs) added by foliar and soil applications during two consecutive seasons.

Year	Treatment	Days taken to first bud initiation	Duration of flowering	Length of flower stalk (cm)	Number of flowers	Flower diameter (cm)	Flower FW (g flower ⁻¹)	Flower DW (g flower ⁻¹)	
2017	Control	120.7 ± 1.15a	55.7 ± 0.58e	7.67 ± 0.06 g	5.67 ± 1.15e	5.43 ± 0.06e	9.38 ± 0.00 h	2.35 ± 0.04f	
	F100	111.3 ± 0.58b	62.0 ± 1.00d	8.60 ± 0.10e	6.33 ± 1.15de	5.93 ± 0.06 cd	11.78 ± 0.02 g	2.86 ± 0.01e	
	F200	109.0 ± 1.00bc	65.3 ± 0.58c	8.93 ± 0.06d	8.33 ± 0.58bcde	6.13 ± 0.06c	12.04 ± 0.03f	3.06 ± 0.01d	
	S200	119.0 ± 1.00a	61.7 ± 1.15d	8.10 ± 0.10f	8.00 ± 1.00cde	5.77 ± 0.06d	12.11 ± 0.01f	3.07 ± 0.01d	
	S400	118.0 ± 0.00a	62.7 ± 1.15d	8.73 ± 0.06de	9.00 ± 1.00abcd	5.93 ± 0.06 cd	12.45 ± 0.12e	3.09 ± 0.02 cd	
	S600	103.0 ± 1.00d	66.0 ± 1.00c	9.63 ± 0.06c	9.33 ± 0.58abc	6.13 ± 0.06c	12.65 ± 0.02d	3.14 ± 0.04 cd	
	F100S200	108.7 ± 1.53bc	61.3 ± 1.53d	9.57 ± 0.06c	9.67 ± 1.15abc	5.97 ± 0.06 cd	12.78 ± 0.05c	3.16 ± 0.05c	
	F100S400	98.0 ± 1.00e	68.7 ± 0.58b	10.33 ± 0.06b	10.33 ± 0.58abc	6.73 ± 0.06b	12.80 ± 0.06bc	3.17 ± 0.06bc	
	F100S600	95.7 ± 0.58ef	69.7 ± 0.58b	10.53 ± 0.06b	10.33 ± 1.15abc	6.87 ± 0.15b	12.91 ± 0.01bc	3.25 ± 0.00b	
	F200S200	106.3 ± 0.58c	61.3 ± 0.58d	9.43 ± 0.06c	10.67 ± 0.58abc	6.87 ± 0.06b	12.90 ± 0.01b	3.25 ± 0.01b	
	F200S400	95.0 ± 1.00f	72.7 ± 0.58a	10.47 ± 0.12b	11.00 ± 1.00ab	6.87 ± 0.06b	12.92 ± 0.02bc	3.26 ± 0.01b	
	F200S600	90.7 ± 0.58 g	73.7 ± 0.58a	11.27 ± 0.12a	11.33 ± 0.58a	7.23 ± 0.12a	13.25 ± 0.03a	3.63 ± 0.02a	
	Control	122.7 ± 0.58a	50.3 ± 0.58i	6.57 ± 0.06 j	5.67 ± 0.58d	4.63 ± 0.06 g	9.66 ± 0.03 h	2.34 ± 0.03f	
	F100	113.0 ± 1.00b	62.0 ± 1.73gh	7.77 ± 0.06i	6.67 ± 0.58 cd	5.83 ± 0.06e	11.82 ± 0.05 g	2.86 ± 0.01e	
2018	F200	112.7 ± 0.58b	66.0 ± 1.00def	8.43 ± 0.06 h	8.33 ± 0.58bc	6.17 ± 0.06 cd	12.06 ± 0.02f	3.05 ± 0.03d	
	S200	121.3 ± 1.53a	63.0 ± 1.00fgh	8.70 ± 0.10 g	8.67 ± 1.15bc	5.50 ± 0.10f	12.25 ± 0.02e	3.08 ± 0.01d	
	S400	120.7 ± 2.08a	64.0 ± 1.00efg	9.03 ± 0.15f	9.33 ± 0.58ab	5.97 ± 0.06de	12.39 ± 0.13e	3.08 ± 0.02d	
	S600	110.3 ± 1.53bc	66.7 ± 0.58de	9.93 ± 0.06d	9.33 ± 0.58ab	6.17 ± 0.06 cd	12.71 ± 0.04d	3.13 ± 0.00d	
	F100S200	111.0 ± 1.00bc	60.7 ± 1.53 h	9.67 ± 0.06e	9.67 ± 1.15ab	6.23 ± 0.06c	12.75 ± 0.03 cd	3.10 ± 0.03d	
	F100S400	99.7 ± 0.58d	62.3 ± 0.58gh	9.87 ± 0.06de	10.33 ± 0.58ab	6.93 ± 0.12b	12.88 ± 0.02bc	3.24 ± 0.01b	
	F100S600	98.3 ± 0.58d	71.3 ± 1.53b	10.70 ± 0.10bc	10.67 ± 0.58ab	6.97 ± 0.12b	12.90 ± 0.01b	3.24 ± 0.01bc	
	F200S200	109.0 ± 1.00c	68.0 ± 1.00 cd	10.47 ± 0.06c	9.33 ± 1.53ab	6.93 ± 0.12b	12.80 ± 0.06bcd	3.14 ± 0.00 cd	
	F200S400	97.0 ± 1.00de	71.0 ± 1.00bc	10.73 ± 0.15b	10.67 ± 0.58ab	6.97 ± 0.12b	12.90 ± 0.01bc	3.24 ± 0.01b	
	F200S600	94.0 ± 1.00e	74.7 ± 0.58a	11.23 ± 0.06a	11.67 ± 0.58a	7.27 ± 0.06a	13.20 ± 0.04a	3.56 ± 0.10a	
	F-test (Two-ways)								
	Year		***	ns	ns	ns	ns	*	*
	Treatment		***	***	***	***	***	***	***
	Year X Treatment		**	***	***	ns	***	***	ns

Data presented are mean ± SD (n = 9).

Means in the same column and within the same year followed by different letters are significant according to Tukey's test (p ≤ 0.05).

The treatment of F200S600 had the longest flower stalk, which was higher than that of control by almost 47 %. Results revealed that the combined addition of SiNPs of both foliar and soil applications has a higher positive effect on the length of flower stalk than the individual application, particularly of the high SiNPs concentrations (Table 3). The results obtained from the second year of the experiment were similar to those reported for the first year without any significant differences.

In the same line with the previous flowering characteristics, the number of flowers positively and significantly responded to the SiNPs application. However, high SiNPs concentrations resulted in a higher number of flowers regardless of the application technique (Table 3). Results also revealed that the foliar application had a little advantage over the soil application when the same SiNPs concentration was used. Upon treating marigold plants with the lowest SiNPs concentration through foliar technique (F100), an increase of 11.6 % in the number of flowers was determined compared to the untreated plants. However, the highest number of flowers (11.33 flowers) linked to the treatment of F200S600 (i.e., the highest applied concentrations of SiNPs in our experiment). The combined treatments of foliar and soil applications had higher numbers of flowers compared to the individual application of SiNPs and control plants (Table 3). No significant differences were statistically calculated between the two years of the experiment, where the results of the second year were consistent with the findings of the first year.

Similar to the number of flowers, the diameter of the flower (cm) of marigold plants significantly affected by the exogenous application of SiNPs added as a foliar, soil application, or their combination. Control plants had the smallest flower diameter (5.43 cm). The diameter of the flower directly proportioned to the addition of SiNPs regardless of the method of application, as all the treated plants had a larger flower diameter. Plants received 200 mg L⁻¹ via the foliar application had a larger flower diameter (6.13 cm) than those received the same concentration of SiNPs but via the soil application (5.77 cm); this gives a slight priority to the foliar application over the soil application (Table 3). The dual application of high SiNPs concentration of foliar and soil applications exhibited larger flower diameter than the individual application except for the treatment of F100S200. However, the largest flower diameter (7.23 cm) corresponded to the treatment of F200S600. Almost the same results were reported to the second year of the experiment, where no significant differences were reported between the two years of the experiment.

The fresh weight of the marigold flower slightly increased upon increasing the dose of SiNPs regardless of the application method. Untreated (control) plants had lowest flower fresh weight (5.43 g flower⁻¹). The soil application of 200 mg L⁻¹ of SiNPs showed a slight priority over the foliar application of the same concentration. The flower fresh weight was 12.11 and 12.04 g flower⁻¹ for soil and foliar application, respectively, of 200 mg L⁻¹ (Table 3). The combination of foliar and soil applications of SiNPs resulted in the highest flower fresh weight (13.25 g flower⁻¹), which is almost 38 % higher than that of control flowers. Similarly, the dry weight of the flower exhibited the same trend and response to the SiNPs application. While the control plants had the lowest flower dry weight (2.35 g flower⁻¹), the highest addition of SiNPs (F200S600) resulted in the highest dry weight of flower (3.63 g flower⁻¹). No significant differences were found between the foliar and soil applications of SiNPs (Table 3). The combined foliar and soil application of SiNPs was better than the singular application. Similar results of fresh and dry weights of flowers were reported in the second growing season; however, the differences between the years were statistically significant.

4. Discussion

In the present study, marigold plants significantly responded to the exogenous application of SiNPs regardless of the application method. Almost all measured vegetative and flower's traits were higher in treated plants than control plants. Several studies, however, have been

reported similar effects of SiNPs on different plant crops such as common bean (*Phaseolus vulgaris*; Alsaeedi et al., 2017b), cucumber (*Cucumis sativus*; Alsaeedi et al., 2019b), maize (*Zea mays* L.; Kaya et al., 2006), wheat (*Triticum aestivum*; Gong et al., 2003), and soybean (*Glycine max*; Hamayun et al., 2010). Despite the positive effect of Si on plant development is well-documented in several plant crops belonging to monocots (Mehrabanjoubani et al., 2015) and dicots (Li et al., 1989), most of the ornamental plants lack such investigation.

The application of SiNPs significantly enlarged the leaf area of the marigold plants as well as enhanced the relative chlorophyll content (SPAD). This might explain the positive effect of SiNPs on growth and development of the marigold plants, where large leaves with high SPAD content have higher rates of photosynthesis process and thus improve the nutritional status. Also, precipitation of Si in the cell wall of leaves corrects the position and orientation of leaves leading to a better light interception and consequently increases photosynthesis rate (Inanaga et al., 1995).

The improvement in the antioxidant capacity of plants treated with SiNPs has been previously introduced as a proposed mechanism inducing plant growth (Jafari et al., 2012). However, in our experiment, we noticed a linear decrease in the activity of CAT and POD upon increasing the dose of applied SiNPs. This might be due to the better growth condition and nutrition status created by SiNPs. Cucumber plants showed lower levels of reactive oxygen species (i.e., H₂O₂), ion leakage, proline content, and peroxidation of bilayer upon the addition of Si compared to control; besides, higher levels of non-enzymatic antioxidants were measured (Jafari et al., 2012). Alsaeedi et al. (2017b) cited that treating common bean with 300 mg L⁻¹ of SiNPs induced the growth recording an increase of 93.5 % and 78.8 % in root and shoot dry weights of common bean, while the root and shoot lengths increased by 32.7 % and 13.2 %, respectively. However, similar increases have been documented in wheat biomass when plants were treated with a low concentration of Si, added as SiO₂, while the higher concentration reduced the shoot biomass (Neu et al., 2017). Similar results were cited by Alsaeedi et al. (2019a) who mentioned that higher SiNPs above 200 mg L⁻¹ reduced the growth of cucumber and resulted in lower fruit yield.

Strikingly, our results were in the same line with those reported above with slight differences. Treating marigold plants with SiNPs, regardless of the concentration and the application method, significantly increased the vegetative parameters. The highest applied SiNPs concentration as combined foliar and soil application (F200S600) resulted in the highest values of plant height, stem diameter, number of branches, root length, root fresh weight, and root dry weight among all the treatments. The Si content in the plants linearly increased upon increasing the concentration of the applied SiNPs in all the application methods. However, Alsaeedi et al. (2019a) reported a linear increase of Si content in cucumber tissues up to 200 mg kg⁻¹ then started to decrease with increasing SiNPs concentration above 200 mg kg⁻¹.

The decrease in Si accumulation in plant tissues, despite the higher applied SiNPs concentration, may be attributed to the saturation state that maintained in root-cell symplast upon the application of high SiNPs concentration (Mitani and Ma, 2005). This supports our hypothesis that the combined foliar and soil application of SiNPs is much better than the singular application in order to avoid the saturation state that may be attended in either root or leaves. In the present study, Si content in plant leaves follows a dose-response relationship, where increasing the concentration of applied SiNPs resulted in more accumulated Si in leaf tissues. The soil application of SiNPs resulted in significantly higher accumulated Si in plant leaves when the same concentration of SiNPs (200 mg L⁻¹) was applied as foliar application. This means plants have higher preference to uptake SiNPs and accumulate Si in their leaves by soil application than foliar application. Yet, the interaction between soil and foliar application resulted in the highest accumulated Si content in the leaves.

Several factors control the success of uptake and distribution of

nanoparticles within plant tissues including plant species, age, and growth conditions as well as characteristics of applied nanoparticles such as particle size, charge, surface area, stability, and the delivery way. Despite foliar application is the common way to deliver nanoparticles to plant, the diameter of pores in the cell wall restricts the penetration of used nanoparticles. Only particles with a diameter of 5–20 nm are easily cross the cell wall based on the diameter of cell wall pores (Navarro et al., 2008). Nair et al. (2010) reported that nanoparticles introduced into plant cells by specific transporter proteins in aquaporin, ion channels, or endocytosis. Also, entering nanoparticles into plant cells could take place through stomata or the base of trichome (Uzu et al., 2010). Uptake nanoparticles through root cells happen by forming complexes with transporters located in the membrane or root exudates (Kurepa et al., 2010). Uptake of Si by plant root undergoes active or passive transportation and many higher plants accumulate substantial amounts of Si in their aboveground part in the form of phytoliths (Epstein, 2009).

Seyfferth and Fendorf (2012) ascribed the enhancement in plant growth to an increase in the phytoavailability of soil phosphorus due to the competition for binding on soil complex between Si and phosphorus. The synergetic effect of Si on the bioavailability of other nutrients to plants has been well-documented for nitrogen (Cuong et al., 2017), potassium (Alsaeedi et al., 2018), iron (Mali and Aery, 2009), phosphorus (Seyfferth and Fendorf, 2012), and zinc (Curie and Briat, 2003). The substantial role of Si in enhancing the nutrient availability extends to include the translocation of macro and microelements in root and shoot parts. Another proposed mechanism for improving plant growth due to Si application is that Si increases the accumulation of Mg in leaf tissue enhancing photosynthesis process (Greger et al., 2018). Also, accumulated Si in the cell wall, as a precipitated thick layer in the epidermal cell (phytoliths), reduces transpiration rate enhancing plant-water relationship (Chen et al., 2018). Also, Si reduces stomatal conductance (Chen et al., 2016).

For ornamental plants, longer flowering periods and shorter time taken for first bud initiation are among the desired traits; in addition to the number of flowers, and diameter of flower. Plant has also several medical and herbal uses especially its lutein (yellow component); therefore, higher fresh and dry weights of flowers are of the importance. In the current study, all the flower traits and flowering period positively and significantly increased upon the addition of SiNPs. The combined application of SiNPs as foliar and soil application exhibited the highest values for the traits mentioned above. This could be attributed to the increase in the accumulated Si in leaves upon the application of higher SiNPs concentration together by foliar and soil application (F200S600). Several studies cited an increase in fruit yield of common bean (Alsaeedi et al., 2017a) and cucumber (Alsaeedi et al., 2019a) as a result of higher contents of Si found in the leaves.

However, the exogenous application of Si to plants becomes very important and crucial for increasing the productivity despite being the second most abundant element in the Earth's crust due to its very low phytoavailability and intensive agricultural programs that remove about 210–240 million tons of Si annually (Meunier et al., 2008). The synergetic effect of Si on the growth and development of plants may be attributed to high relative water content, upregulation of phytohormones, induced cell elongation, improved uptake of nutrients, high activity of antioxidant enzymes, maintained membrane integrity, and enhanced structure of chloroplast (Liang et al., 2006; Alsaeedi et al., 2019a).

5. Conclusion

The dual application of SiNPs to the plants as foliar and soil applications improved not only the plant biometrics and plant physiology but also enhanced the flower's characteristics and flowering period. However, this potential impact of SiNPs on plant growth and flowers is attributed to the enhancement in the plant-water relationship, nutrient

uptake, cell elongation, photosynthetic pigments, and antioxidant capacity. Although the individual application by either foliar or soil (drench) application had a positive effect on plant growth, the most important effect was ascribed to the combined foliar and soil application that linearly increased the plant tissue Si content. Significantly and interestingly, the exogenous application of SiNPs as a combined foliar and soil application resulted in higher SPAD content, leaf area, number of flowers, a shorter period of the initiation of the first bud, longer flowering period and higher fresh and dry weight of flowers.

Data availability

All data are fully available without restriction.

CRediT authorship contribution statement

Eman A. Attia: Conceptualization, Methodology, Software, Supervision. **Neveen Elhawaw:** Methodology, Software, Data curation, Writing - original draft, Visualization, Investigation, Software, Validation, Writing - review & editing.

Declaration of Competing Interest

We have no conflicts of interest to disclose.

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