SILICON DIOXIDE NANOPARTICLES, ELICITATION OF SILYBIN CONCENTRATION IN *SILYBUM MARIANUM* L. THROUGH CHALCONE SYNTHASE GENE EXPRESSION MODIFICATION

ALMULHIM, B. F.[1] – EL SHERIF, F.[1,2*] – KHATTAB, S. [1,2]

[1]*Department of Biological Sciences, College of Science, King Faisal University, AlAhsa 31982, Saudi Arabia*
(e-mails: balmulheml@kfu.edu.sa, Skhattab@kfu.edu.sa)

[2]*Department of Horticulture, Faculty of Agriculture, Suez Canal University, Ismalia 41522, Egypt*

***Corresponding author**
e-mail: Felsherif@kfu.edu.sa; phone: +966-53-5541-288

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Abstract. Using nanoparticles in plants has been demonstrated to increase growth and output while also altering the ratio of bioactive compounds in numerous plant species. An herb known as *Silybum marianum* synthesizes silymarin, a flavonolignan with liver-protective properties. Silymarin or its primary elements, silybin A and B, which are present mainly in *S. marianum* fruits, have been the subject of many attempts to increase its manufacture. Various quantities of SiO_2 NPs were used as a spray on the foliage of *S. marianum* crops grown in a greenhouse in order to study the impact of silicon dioxide nanoparticles (SiO_2 NPs) on growth, yield, photosynthetic pigment content, silybin A + B content, and chalcone synthase (CHS) genes expression. The amounts of bioactive components (silybin A + B) in *S. marianum* rose after foliar application of all tested SiO_2 NP_S doses, improving plant growth and yield as well. Additionally, it was shown by the gene expression study that SiO_2 NPs increased silybin (A + B) production by stimulating CHS genes. The most effective SiO_2 dosage that improved yield and silybin content was 100 mgl^{-1}.
Keywords: *milk thistle, silymarin, HPLC, RT-PCR, nano fertilizer*

Introduction

One of the most significant uses of nanotechnology for farming is the use of nanofertilizers, which can feed plants steadily and under controlled circumstances, unlike conventional fertilizers. Quick absorption, progressive discharge, and the use of nanofertilizers in modest doses can all help to lessen pollution in the environment (Liu and Lal, 2015). Silicon (Si) is known to help crops by regulating a number of physiological processes (Parveen and Ashraf, 2010; Luyckx et al., 2017; Siddiqui et al., 2018). Silicon dioxide (SiO_2) has distinct physicochemical properties that enable them to infiltrate crops, change metabolism, and enhance yield and development in unfavourable environmental conditions (Cai et al., 2009; Balakhnina and Borkowska, 2013; Ye et al., 2013; Rizwan et al., 2015; Luyckx et al., 2017; Wang et al., 2017). A member of the Asteraceae family, milk thistle (*Silybum marianum* L.) is an annual winter or biennial plant native to the Mediterranean region (Sedghi et al., 2010). The most significant secondary metabolite of *S. marianum* is silymarin, which is present in every part of the plant with varying amounts but is more abundant in the seed and fruit (Biedermann et al., 2014; El-Garhy et al., 2016). Silymarin is used in many medicines and nutraceutical items because of its hepatoprotective properties, but it also has numerous of other

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pharmacological properties, such as cardioprotective, hypocholesterolemic, and anti-diabetic properties (Škottová and Krečman,1998; Matsuda et al., 2005; Abenavoli et al., 2010; Fallahzadeh et al., 2012; Zholobenko and Modriansky, 2014). At least ten biogenetic congeners with structurally similar flavonolignans make up silymarin (Chambers et al., 2017). The silymarin component known as silybin has enormous potential as a cancer and Alzheimer's disease treatment (Křenek et al., 2014; Sciacca et al., 2017). In *S. marianum* and other fungi, plants, and microorganisms, the chalcone synthase enzyme (CHS) family has been found to play a significant role in the synthesis of flavonolignans (Sanjari et al., 2015; Lv et al., 2017; Kaur et al., 2020; Wu et al., 2020). To satisfy the rising requirement from both the pharmaceutical sector and indigenous medicinal systems, commercial production of *S. marianum* crops is necessary. We investigated how SiO_2 nanoparticles influenced the growth, yield, and chemical composition of *S. marianum* crops grown in greenhouses. To better understand the molecular processes underlying the reactions to SiO_2 NPs methods, the quantitative reverse-transcribed polymerase chain reaction (qRT-PCR) was used to characterize the expression trends of the chal-cone synthase 1 (CHS 1), 2 (CHS 2), and 3 (CHS 3) genes.

Materials and methods

Plant materials and growth conditions

The study was carried out at the Agricultural and Veterinary Research and Training Center of the King Faisal University, the study was carried out in a greenhouse in 2019. 1st October 2019 seeds of *S. marianum* were acquired from King Faisal University's Agriculture and Veterinary Research and Training Center in Saudi Arabia. Sand-filled germination trays were used to hold *S. marianum* seeds. (at a depth of 1.0-2.0 cm). *S. marianum* saplings were with 80×80 cm distance in sandy soil, and transplanted after 30 days when they were 5 cm high and had three real leaves. The crops were watered using drip irrigation, with drippers 80 cm separated along the irrigation pipe. The flow rate was kept at 4.0 L/h on average. Three levels of SiO_2 NPs (catalogue no. 637246) with a mean particle size of 5–15 nm were used as groups for treatment in the studies, with distilled water serving as the control. Each concentration (10.0, 50.0, and 100.0 mgl^{-1}) (Adhikari et al.,2013) had 20 plants as replicated. SiO_2 NPs solutions were applied to the whole foliage at periods of two months following an 8-week soil-culture period. The entire foliage of each plant were sprayed with around 50 mL of SiO_2 NPs solutions in the morning at two-months interval. *Tables A1* and *A2* include the analysis of the soil and irrigation water components (Buurman et al.,1996).

The following parameters were evaluated 30 weeks after sowing in six randomly chosen plants from every treatment group: height of the plant (cm), the number of branches/plant, number of leaves/plant (n), and the dry weights (g) of root and the aerial part. Whenever they were ready, mature fruits were regularly harvested from every plant and allowed to air dry. The number of capitula (n) per plant as well as the dried weight (g) of the fruits were recorded.

Chemical analysis

Measurement of photosynthetic pigment in fresh leaves

The third bottom new leaves of six randomly chosen 27-week-old *S. marianum* plants were removed. Using 80% acetone as the solvent, the concentrations of

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carotenoid and chlorophyll a and b (Chl. a, Chl. b) were measured, as explained by (Williams, 1984). An Agilent 8453 ultraviolet (UV) visible spectrophotometer was used to calculate the absorbance.

Analysis of nitrogen, phosphorus, potassium and silicon composition in S. marianum dried leaves, roots, and fruits

Dried leaf, root, and fruit specimens were separately smashed and sulphuric acid digested using the (Cottenie et al., 1980) method.

Nitrogen (N) was measured using the altered micro- Kjedahl method, as explained by Jackson (1967), According to the technique of colorimetry, the percentage of phosphorus (P) was measured (Murphy and Riley, 1962), and using a Shimadzu-AA7000 Atomic Absorption Flame Photometry model, potassium (K) concentrations (%) were calculated using (Mazumdar and Majumder, 2003). Si content was assessed using the technique of Frantz et al. (2008).

High-performance liquid chromatography (HPLC) method for determination of silybin (A + B) in the dry fruits

Using a Shimadzu LC-2030 system (Shimadzu, Ni-shinokyo, Japan) fitted with a C18 monolithic column (4.650 mm) array detector, the silybin (A + B) concentration of air-dried fruit samples for every course of therapy was measured (Yap et al., 2021). Authentic standards of silybin A + B (93.06%) (cat. no. S465850) were purchased from Toronto Research Chemicals, Canada. A silybin A + B stock solution of 1 mg ml^{-1} in methanol was prepared and diluted to obtain standard solutions of 50, 100, 250, 500, 600, and 700 μg ml^{-1}. Each of the dilutions was filtered using a 0.45-μm syringe filter, and 5.0 μl was injected. The extracts were prepared from the dried fruit samples by methanol/dichloromethane (1:1, v:v) extraction. Dried fruit powder (1 g) was mixed with 20 mL of solvent and left overnight. The solvent was collected and replaced with the same amount of fresh solvent every day for three consecutive days to ensure complete extraction. The solvents from the collected filtrates were evaporated in a fume hood to obtain the dry residue for each sample. For the HPLC analysis, a known weight of the residue was dissolved in 5 ml of the mobile phase [0.25% acetic acid in acetonitrile and water (v/v 90:10)] in a volumetric flask. The contents of each flask were shaken vigorously for 10 min, then sonicated for 15 min before filtration through 0.45-μm disposable filters. Before injection, the sample was filtered with a 0.22-μm syringe filter. A sample of 5.0 μL was then injected. The HPLC separation and quantitation were performed with a Column C18 monolithic column (4.6×50 mm). The mobile phase [0.25% acetic acid in acetonitrile and water (v/v 90:10)] was prepared. The mobile phase was filtered using a 0.45-μm membrane filter (Millipore, Milford, MA) and degassed by vacuum prior to use. The flow rate was set at 1 ml/min. All determinations were performed at ambient temperature (25°C), with UV detection at 280 nm.

Gene expression analyses of chalcone synthase 1, 2, and 3 by real-time re-verse-transcribed Polymerase chain reaction (real-time RT-PCR)

Six *S. marianum* plants from each treatment group had their petals from chalcone synthase 1, 2, and 3 genes analysed using gene-specific primers (*Table A3*). Real-time RT-PCR, total RNA extraction, cDNA synthesis, and petal specimen gathering and preservation were all completed as per with (Crocenzi and Roma,2006).

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Statistical analysis

An entirely random block structure with six repeats was used in the research. The results from every calculation in Statistica 6 were evaluated using ANOVA (StatSoft, 2001; www.statistica.com). At a probability level of $p = 0.05$, the mean difference between treatment groups was determined. The LSD test was utilized to assess the significance of mean differences.

Results

Plant growth and yield

SiO_2 NPs (10.0, 50.0, and 100 mgl^{-1}) significantly enhanced plant height, the number of leaves, the number of branches, the dry weight of aerial parts, and the dry weight of roots in the *S. marianum* plant compared to control treatment (*Table 1*). *Table 2* shows the impact of SiO_2 NP levels on *S. marianum* yield as determined by capitula number and fruit weight when dry. The afore-mentioned characteristics were substantially enhanced at all SiO_2 NP levels when compared with the control treatment (*Table 2*).

Table 1. *Effect of SiO_2 NPs concentrations on plant height, number of leaves, branch number, aerial part dry weight, and root dry weight of S. marianum plants*

SiO_2 NPs (mgl^{-1})	Plant height (cm)	Number of leaves (n)	Branch number (n)	Aerial part dry weight (g)	Root dry weight (g)
Control	87.83 b	24.00 d	3.83 c	52.380 c	6.01 b
10.0	101.67 a	113.83 a	9.67 b	171.135 a	11.99 a
50.0	98.33 a	106.50 b	8.67 b	131.187 b	12.08 a
100.0	96.00 a	96.67 c	10.50 a	159.085 ab	13.29 a

Means followed by the same letter within a column are not significantly different at the 0.05 level of probability according to the least significant difference (LSD) test

Table 2. *Effect of SiO_2 NP concentrations on capitula number and fruit dry weight (g) of S. marianum plants*

SiO_2 NPs (mgl^{-1})	Capitula number (n)	Fruit dry weight (g)
Control	5.33 b	8.197 b
10.0	9.67 a	26.533 a
50.0	8.67 a	34.510 a
100.0	10.50 a	31.468 a

Means followed by the same letter within a column are not significantly different at the 0.05 level of probability according to the least significant difference (LSD) test

Chemical composition

Photosynthetic pigment in leaves

Table 3 shows that, in comparison to the control treatment, all SiO_2 NPs concentrations considerably significantly enhanced the Chl. b contents in the leaves of *S. marianum*. In comparison to the control (6.804 mg/100 g FW), Chl. b reached a maximum value of 12.751 mg/100 g FW at a SiO_2 NPs level of 100.0 mgl^{-1}. The effects of Chl. a and carotenoids changed depending on the concentration of SiO2 NPs (*Table 3*).

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Table 3. *Effect of SiO_2 NPs concentrations on chlorophyll a (Chl. a), chlorophyll b (Chl. b) and carotenoids of S. marianum plants*

SiO_2 NPs (mgl^{-1})	Chl a (mg/100 g F.W.)	Chl b (mg/100 g F.W.)	Carotenoids (mg/100 g F.W.)
Control	26.065 a	6.804 b	25.103 b
10.0	24.200 b	9.331 a	24.749 b
50.0	28.356 ab	11.436 a	28.871 ab
100.0	33.257 a	12.751 a	34.5453 a

Means followed by the same letter within a column are not significantly different at the 0.05 level of probability according to the least significant difference (LSD) test

Mineral elements contents in leaves, roots, and fruits

Tables 4, 5, and *6* indicate the effects of SiO_2 NPs on nitrogen (N), phosphorus (P), potassium (K), and silicon (Si), with an emphasis on the leaves, roots, and fruits of *S. marianum* crops. When compared to control treatments, N and P levels in crop leaves reduced as SiO_2 NP levels increased, but K and Si levels increased (*Table 4*). As opposed to the controls, all SiO_2 NPs increased the N, P, K, and Si contents in the roots of the SiO_2 NPs crop (*Table 5*). SiO_2 NPs at 50.0 mgl^{-1} had the greatest N and Si elements, whereas those at 100.0 mgl^{-1} generated the greatest P and K elements (*Table 5)*. The *S. marianum* fruits showed similar outcomes, and all of the SiO2 NP treatments considerably increased the N and P contents relative to the control treatment (*Table 6*). At 100.0 mgl^{-1} SiO_2 NPs, the greatest N and P contents (2.34 and 1.33%, respectively) were attained. SiO_2 NPs at 100.0 mgl^{-1} considerably enhanced the K content in plant fruits, while the K content of *S. marianum* fruit dropped at the other doses when compared to the control treatment (*Table* 6). SiO_2 NPs at 10.0 and 50.0 mgl^{-1} raised the Si content in the fruits relative to the control treatment; however, the high Si level lowered this parameter relative to the control and the other SiO_2 NPs levels in the plant's fruits (*Table 6*).

Table 4. *Effect of SiO_2 NPs concentrations on nitrogen, phosphorus, potassium, and silicon compositions in the leaves of S. marianum plants*

SiO_2 NPs (mgl^{-1})	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Silicon (ppm)
Control	1.145 a	0.698 a	1.725 b	1.408 c
10.0	0.780 b	0.369 c	2.125 a	1.805 a
50.0	0.770 b	0.577 b	2.145 a	1.730 b
100.0	0.995 a	0.555 b	1.960 b	1.885 a

Means followed by the same letter within a column are not significantly different at the 0.05 level of probability according to the least significant difference (LSD) test

HPLC analysis of silybin (A + B)

Figure 1 displays the silybin concentrations (A + B). The findings showed that, in comparison to the control treatment, increasing SiO_2 NP levels considerably raised the silybin (A + B) contents of *S. marianum* fruits. Crops sprayed with SiO_2 NPs had the greatest silybin (A + B) content, 100.0 mgl-1 (*Fig. 1*).

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Table 5. *Effect of SiO_2 NPs concentrations on nitrogen, phosphorus, potassium, and silicon compositions in the roots of S. marianum plants*

SiO_2 NPs (mgl^{-1})	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Silicon (ppm)
Control	0.890 b*	0.794 c	2.18 b	0.74 b
10.0	0.950 a	0.989 b	2.71 a	1.70 ab
50.0	1.055 a	1.017 ab	2.25 ab	1.97 a
100.0	1.045 a	1.045 a	2.29 ab	1.40 ab

Means followed by the same letter within a column are not significantly different at the 0.05 level of probability according to the least significant difference (LSD) test

Table 6. *Effect of SiO_2 NPs concentrations on nitrogen, phosphorus, potassium, and silicon compositions in the fruits of S. marianum plants*

SiO_2 NPs (mgl^{-1})	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Silicon (ppm)
Control	1.90 b*	1.07 d	1.65 b	1.21 bc
10.0	2.19 a	1.27 c	1.45 b	1.39 a
50.0	2.08 a	1.30 b	1.45 b	1.28 ab
100.0	2.34 a	1.33 a	2.07 a	1.11 c

Means followed by the same letter within a column are not significantly different at the 0.05 level of probability according to the least significant difference (LSD) test

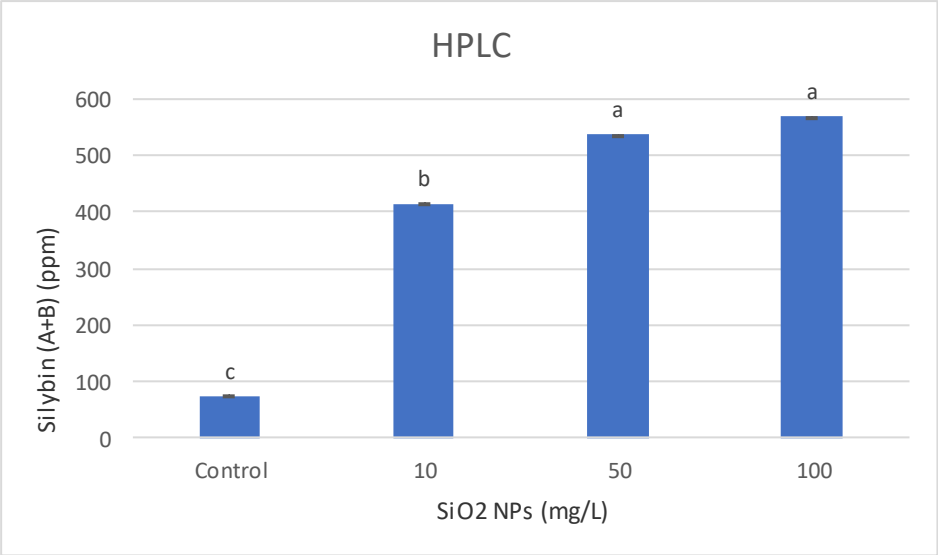

Figure 1. *Effect of SiO_2 NP concentrations on silybin (A + B) content in the fruits of S. marianum plants*

The effect of SiO2 NPs on CHS 1, 2, and 3 gene expression

The CHS1, -2, and -3 genes were examined using qRT-PCR to investigate how different dosages of SiO_2 NPs altered their expression levels (*Fig. 2*). SiO_2 NPs foliar spray increased CHS1, -2, and -3 expressions of genes, with CHS1 showing the maximum level of expression in the treatment groups that received 100 mgl^{-1} and 50

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mgl^{-1} of SiO_2 NPs foliar spray, respectively. While CHS 2's level of expression has experienced the opposite phenomenon. The 50 mgl^{-1} SiO_2 NPs foliar spray treatment produced the highest amount of CHS3 gene expression, followed by the 10 mgl^{-1} SiO_2 NPs foliar spray treatment (*Fig. 2*). This result is consistent with our morphological data, which demonstrated that the SiO_2 NPs treatment group enhanced plant growth, yield, and silybin (A + B) content.

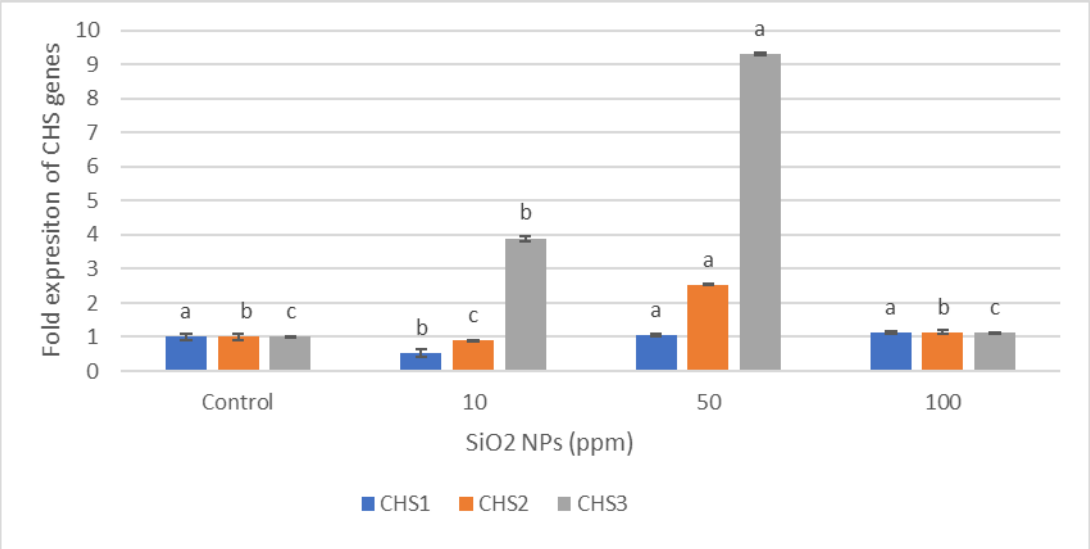

Figure 2. *Fold changes in CHS1, -2, and -3 expressions as a result of the SiO_2 NPs treatment. For each treatment group, four biological replicates were used to calculate the means and standard deviations. For each biological replicate, duplicate PCR was run*

Discussion

The impact of SiO_2 NPs on *S. marianum* plants' growth, production, capacity for photosynthesis, and levels of N, P, K, Si, and silybin (A + B) as well as the expression of the CHS 1, 2, and 3 genes were examined in this study. Plant height, leaf count, branch count, aerial part and root dry weight, and growth parameters of plants treated with different levels of SiO_2 NPs were all measured. When compared to control plants, the application of different SiO_2 NP levels boosted all of the aforementioned traits. Increased plant growth in the presence of Si NPs has previously been documented (Hassanpour et al., 2020; Attia and Elhawat, 2021; Fatemi et al., 2021; Ferrusquia-Jimenez et al.,2022; Mathur and Srivastava,2022; Thakur et al.,2022; Parveen and Siddiqui, 2022). The amount of shoot length and leaf growth appeared to be mostly influenced by the amount of NPs applied (Hassanpour et al., 2020; Zafar et al., 2016). In this study, increasing SiO_2 NPs concentrations from 0 to 10.0 mgl^{-1} resulted in steady increases in plant height and leaf number. These values fell above 10.0 mgl^{-1}, but remained higher than in the controls. Increased shoot length was recently observed under varied concentrations of SiO_2 NPs (Ferrusquia-Jimenez et al., 2022; Parveen and Siddiqui, 2022), which is consistent with the present results. The magnitude of the increase in shoot length and leaf number appeared to be mostly determined by the NP concentration employed (Hassanpour et al., 2020; Thakur et al., 2022). Plant growth may have increased in the presence of NPs due to the nutritional behavior of particles or dissociated ions at nonlethal concentrations (Hassanpour et al.,

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2020; Zafar et al., 2016). With increased SiO_2 NP level, *S. marianum* plants produced more yield (in terms of capitula number and dry fruit weight), which was related to better plant growth (Yassen et al., 2017; Attia and Elhawat, 2021; de Souza Junior et al., 2021; Fatemi et al., 2021).

The measuring of photosynthetic pigments is a low-cost, rapid technique that could be beneficial in assessing the fitness of plant growth and yield in the presence of NPs. Because of the strong relationship between this variable and Si accumulation, foliar application of SiO_2 NPs improved Si accumulation in *S. marianum* leaves, roots, and fruits, resulting in advantages in pigment synthesis, photosynthetic parameters, growth, and yield. Increased pigment production was also reported as a benefit of Si. This could be because Si, once absorbed, creates changes in leaf design, with more upright leaves improving solar absorption and photosynthesis and raising chlorophyll and carotenoid creation (Raven, 1983). Foliar application of soluble SiO_2 NPs increased Si accumulation and dry-mass production in a variety of plant species (Raven, 1983; Prakash et al., 2011; Shwethakumari and Prakash, 2018; Pereira de Souza Junior et al., 2019; de Souza Junior et al., 2021; Jeer et al., 2021).

When varied SiO_2 NP concentrations were applied, the N and P contents of the *S. marianum* plant's leaves declined, whereas they grew in the plant's roots and fruits. The application of SiO_2 NPs raised the K content in the leaves and roots. The current study demonstrated that the dose affected the SiO_2 NPs' ability to enhance K ions. A foliar application of SiO_2 NPs improved the percentage of nutrients N, P, and K absorbed in the shoots, roots, and fruits of many crops (Yassen et al., 2017; Chen et al., 2018; Alsaeedi et al., 2019).

SiO_2 NPs application increased the amounts of silybin (A + B) in *S. marianum* fruits, supporting the idea that these compounds are crucial for promoting plant growth. SiO_2 NPs have been observed to induce the biosynthesis of phenolic and flavonoid compounds (Suriyaprabha et al., 2012; Shallan et al., 2016; EL-Kady et al., 2017; Ahmad et al., 2020; Hassanpour et al., 2020). Flavonoid biosynthesis, including the silybin composition, has been connected to the expression of *S. marianum*'s CHS1, -2, and -3 genes (Lv et al., 2017). CHS 1, -2, and -3 expression levels were increased in SiO2 NPs at 50 and 100 mgl^{-1} concentrations. A number of studies have identified the CHS1, -2, and -3 genes from *S. marianum* involved in the silymarin production pathway using diverse techniques (Sánchez-Sampedro et al., 2005; El-Garhy et al., 2016). In addition, the current investigation found a strong correlation between silybin (A + B) concentrations and the expression of the three chalcone synthase (CHS1, -2, and -3) genes.

Conclusions

In this study, the effects of three different SiO_2 NP levels (10, 50, and 100 mgl-1) on the development, yield, silybin (A + B) content, and CHS 1, 2, and 3 expression of genes of *S. marianum* were investigated. By increasing the quantities of bioactive components (silybin A + B), foliar application of all examined SiO_2 NP_S concentrations increased plant development and yield as well as the medicinal value of *S. marianum*. SiO_2 NP_S boosted silybin (A + B) production via stimulating CHS genes, according to gene regulation research. The level of 100 mgl^{-1} SiO_2 NP_S exhibited the best boosting effects on yield and silybin content out of all the SiO_2 NP_S levels tested.

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Conflicts of interests. The authors declare there are no conflicts of interests.

REFERENCES

[1] Abenavoli, L., Capasso, R., Milic, N., Capasso, F. (2010): Milk thistle in liver diseases: past, present, future. – Phytotherapy Research 24(10): 1423-1432.
[2] Adhikari, T., Kundu, S., Rao, A. S. (2013): Impact of SiO2 and Mo nano particles on seed germination of rice (Oryza sativa L.). – Int J Agric Food Sci Technol 4(8): 809-816.
[3] Ahmad, B., Khan, M. M. A., Jaleel, H., Shabbir, A., Sadiq, Y., Uddin, M. (2020): Silicon nanoparticles mediated increase in glandular trichomes and regulation of photosynthetic and quality attributes in Mentha piperita L. – Journal of Plant Growth Regulation 39: 346-357.
[4] Alsaeedi, A., El-Ramady, H., Alshaal, T., El-Garawany, M., Elhawat, N., Al-Otaibi, A. (2019): Silica nanoparticles boost growth and productivity of cucumber under water deficit and salinity stresses by balancing nutrients uptake. – Plant Physiology and Biochemistry 139: 1-10.
[5] Attia, E. A., Elhawat, N. (2021): Combined foliar and soil application of silica nanoparticles enhances the growth, flowering period and flower characteristics of marigold (Tagetes erecta L.). – Scientia Horticulturae 282: 110015.
[6] Balakhnina, T., Borkowska, A. (2013): Effects of silicon on plant resistance to environmental stresses. – International Agrophysics 27(2).
[7] Biedermann, D., Vavříková, E., Cvak, L., Křen, V. (2014): Chemistry of silybin. – Natural Product Reports 31(9): 1138-1157.
[8] Buurman, P., Van Lagen, B., Velthorst, E. J. (1996): Manual for Soil and Water Analysis. – Backhuys, Leiden.
[9] Cai, K., Gao, D., Chen, J., Luo, S. (2009): Probing the mechanisms of silicon-mediated pathogen resistance. – Plant Signaling & Behavior 4(1): 1-3.
[10] Chambers, C. S., Holečková, V., Petrásková, L., Biedermann, D., Valentová, K., Buchta, M., Křen, V. (2017): The silymarin composition… and why does it matter??? – Food Research International 100: 339-353.
[11] Chen, R., Zhang, C., Zhao, Y., Huang, Y., Liu, Z. (2018): Foliar application with nano-silicon reduced cadmium accumulation in grains by inhibiting cadmium translocation in rice plants. – Environmental Science and Pollution Research 25: 2361-2368.
[12] Cottenie, A., Camerlynck, R., Verloo, M., Dhaese, A. (1980): Fractionation and determination of trace elements in plants, soils and sediments. – In: 27th International Congress of Pure and Applied Chemistry. Plenary and Invited Lectures Presented at the 27th IUPAC Congress, Helsinki, Finland, 27-31 August 1979, pp. 45-53. https://doi.org/10.1016/B978-0-08-023936-1.50007-1.
[13] Crocenzi, F. A., Roma, M. G. (2006): Silymarin as a new hepatoprotective agent in experimental cholestasis: new possibilities for an ancient medication. – Current Medicinal Chemistry 13(9): 1055-1074.

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[14] de Souza Junior, J. P., de Mello Prado, R., Soares, M. B., da Silva, J. L. F., de Farias Guedes, V. H., dos Santos Sarah, M. M., Cazetta, J. O. (2021): Effect of different foliar silicon sources on cotton plants. – Journal of Soil Science and Plant Nutrition 21: 95-103.
[15] El-Garhy, H. A., Khattab, S., Moustafa, M. M., Abou Ali, R., Azeiz, A. Z. A., Elhalwagi, A., El Sherif, F. (2016): Silybin content and overexpression of chalcone synthase genes in Silybum marianum L. plants under abiotic elicitation. – Plant Physiology and Biochemistry 108: 191-202.
[16] El-Kady, M. E., El-Boray, M. S., Shalan, A. M., Mohamed, L. M. (2017): Effect of silicon dioxide nanoparticles on growth improvement of banana shoots in vitro within rooting stage. – Journal of Plant Production 8(9): 913-916.
[17] Fallahzadeh, M. K., Dormanesh, B., Sagheb, M. M., Roozbeh, J., Vessal, G., Pakfetrat, M., Daneshbod, Y., Kamali-Sarvestani, E., Lankarani, K. B. (2012): Effect of addition of silymarin to renin-angiotensin system inhibitors on proteinuria in type 2 diabetic patients with overt nephropathy: a randomized, double-blind, placebo-controlled trial. – American Journal of Kidney Diseases 60(6): 896-903.
[18] Fatemi, H., Esmaiel Pour, B., Rizwan, M. (2021): Foliar application of silicon nanoparticles affected the growth, vitamin C, flavonoid, and antioxidant enzyme activities of coriander (Coriandrum sativum L.) plants grown in lead (Pb)-spiked soil. – Environmental Science and Pollution Research 28: 1417-1425.
[19] Ferrusquía-Jiménez, N. I., González-Arias, B., Rosales, A., Esquivel, K., Escamilla-Silva, E. M., Ortega-Torres, A. E., Guevara-González, R. G. (2022): Elicitation of bacillus cereus-amazcala (Bc-A) with SiO2 nanoparticles improves its role as a plant growth-promoting bacteria (PGPB) in Chili pepper plants. – Plants 11(24): 3445.
[20] Frantz, J. M., Locke, J. C., Datnoff, L., Omer, M., Widrig, A., Sturtz, D., Horst, L., Krause, C. R. (2008): Detection, distribution, and quantification of silicon in floricultural crops utilizing three distinct analytical methods. – Communications in Soil Science and Plant Analysis 39(17-18): 2734-2751.
[21] Hassanpour, H., Ahmadi, N., Hekmati, M., Ghanbarzadeh, M. (2020): Effect of SiO2 nanoparticles on phytochemical and anatomical alterations in Anthemis gilanica. – Iranian Journal of Plant Physiology 10(3): 3223-3231.
[22] Jackson, M. (1967): Soil Chemical Analysis. – Prentice Hall of India Private Limited, New Delhi.
[23] Jeer, M., Yele, Y., Sharma, K. C., Prakash, N. B. (2021): Exogenous application of different silicon sources and potassium reduces pink stem borer damage and improves photosynthesis, yield and related parameters in wheat. – Silicon 13(3): 901-910.
[24] Kaur, R., Aslam, L., Kapoor, N., Mahajan, R. (2020): Identification and comparative expression analysis of chalcone synthase, flavanone 3-hydroxylase and dihydroflavonol 4-reductase genes in wild pomegranate (Punica granatum L.) organs. – Brazilian Journal of Botany 43: 883-896.
[25] Křenek, K., Marhol, P., Peikerová, Ž., Křen, V., Biedermann, D. (2014): Preparatory separation of the silymarin flavonolignans by Sephadex LH-20 gel. – Food Research International 65: 115-120.
[26] Liu, R., Lal, R. (2015): Potentials of engineered nanoparticles as fertilizers for increasing agronomic productions. – Science of the Total Environment 514: 131-139.
[27] Luyckx, M., Hausman, J. F., Lutts, S., Guerriero, G. (2017): Silicon and plants: current knowledge and technological perspectives. – Frontiers in Plant Science 8: 411.
[28] Lv, Y., Gao, S., Xu, S., Du, G., Zhou, J., Chen, J. (2017): Spatial organization of silybin biosynthesis in milk thistle [Silybum marianum (L.) Gaertn]. – The Plant Journal 92(6): 995-1004.
[29] Mathur, P. G. J., Srivastava, N. (2022): Silica nanoparticles as novel sustainable approach for plant growth and crop protection. – Heliyon. DOI: 10.1016/j.heliyon.2022.e09908.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2201_681693

[30] Matsuda, T., Ferreri, K., Todorov, I., Kuroda, Y., Smith, C. V., Kandeel, F., Mullen, Y. (2005): Silymarin protects pancreatic β-cells against cytokine-mediated toxicity: implication of c-Jun NH2-terminal kinase and janus kinase/signal transducer and activator of transcription pathways. – Endocrinology 146(1): 175-185.
[31] Mazumdar, B. C., Majumder, K. (2003): Methods on Physico-Chemical Analysis of Fruits. – Daya Publishing House, Delhi, pp. 137-138.
[32] Murphy, J., Riley, J. P. (1962): A modified single solution method for the determination of phosphate in natural waters. – Analytica Chimica Acta 27: 31-36.
[33] Parveen, A., Siddiqui, Z. A. (2022): Impact of silicon dioxide nanoparticles on growth, photosynthetic pigments, proline, activities of defense enzymes and some bacterial and fungal pathogens of tomato. – Vegetos 35: 83-93.
[34] Parveen, N., Ashraf, M. (2010): Role of silicon in mitigating the adverse effects of salt stress on growth and photosynthetic attributes of two maize (Zea mays L.) cultivars grown hydroponically. – Pak J Bot 42(3): 1675-1684.
[35] Pereira de Souza Junior, J., de Mello Prado, R., Machado dos Santos Sarah, M., Felisberto, G. (2019): Silicon mitigates boron deficiency and toxicity in cotton cultivated in nutrient solution. – Journal of Plant Nutrition and Soil Science 182(5): 805-814.
[36] Prakash, N. B., Chandrashekar, N., Mahendra, C., Patil, S. U., Thippeshappa, G. N., Laane, H. M. (2011): Effect of foliar spray of soluble silicic acid on growth and yield parameters of wetland rice in hilly and coastal zone soils of Karnataka, South India. – Journal of Plant Nutrition 34(12): 1883-1893.
[37] Raven, J. A. (1983): The transport and function of silicon in plants. – Biological Reviews 58(2): 179-207.
[38] Rizwan, M., Ali, S., Ibrahim, M., Farid, M., Adrees, M., Bharwana, S. A., Zia-ur-Rehman, M., Qayyum, M. F., Abbas, F. (2015): Mechanisms of silicon-mediated alleviation of drought and salt stress in plants: a review. – Environmental Science and Pollution Research 22: 15416-15431.
[39] Sánchez-Sampedro, M. A., Fernández-Tárrago, J., Corchete, P. (2005): Yeast extract and methyl jasmonate-induced silymarin production in cell cultures of Silybum marianum (L.) Gaertn. – Journal of Biotechnology 119(1): 60-69.
[40] Sanjari, S., Shobbar, Z. S., Ebrahimi, M., Hasanloo, T., Sadat-Noori, S. A., Tirnaz, S. (2015): Chalcone synthase genes from milk thistle (Silybum marianum): isolation and expression analysis. – Journal of Genetics 94: 611-617.
[41] Sciacca, M. F., Romanucci, V., Zarrelli, A., Monaco, I., Lolicato, F., Spinella, N., Galati, C., Grasso, G., D'Urso, L., Romeo, M., Diomede, L. (2017): Inhibition of Aβ amyloid growth and toxicity by silybins: the crucial role of stereochemistry. – ACS Chemical Neuroscience 8(8): 1767-1778.
[42] Sedghi, M., Nemati, A., Amanpour-Balaneji, B., Gholipouri, A. (2010): Influence of different priming materials on germination and seedling establishment of milk thistle (Silybum marianum) under salinity stress. – World Applied Sciences Journal 11(5): 604-609.
[43] Shallan, M. A., Hassan, H., Namich, A. A., Ibrahim, A. A. (2016): Biochemical and physiological effects of TiO2 and SiO2 nanoparticles on cotton plant under drought stress. – Research Journal of Pharmaceutical, Biological and Chemical Sciences 7(4): 1540-1551.
[44] Shwethakumari, U., Prakash, N. B. (2018): Effect of foliar application of silicic acid on soybean yield and seed quality under field conditions. – Journal of the Indian Society of Soil Science 66(4): 406-414.
[45] Siddiqui, H., Yusuf, M., Faraz, A., Faizan, M., Sami, F., Hayat, S. (2018): 24-Epibrassinolide supplemented with silicon enhances the photosynthetic efficiency of Brassica juncea under salt stress. – South African Journal of Botany 118: 120-128.
[46] Škottová, N., Krečman, V. (1998): Silymarin as a potential hypocholesterolaemic drug. – Physiol Res 47(1): 1-7.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2201_681693

[47] STATSOFT (2001): Statistica for Windows, Version 6.0. – STATSOFT, Tulsa, OK.
[48] Suriyaprabha, R., Karunakaran, G., Yuvakkumar, R., Prabu, P., Rajendran, V., Kannan, N. (2012): Growth and physiological responses of maize (Zea mays L.) to porous silica nanoparticles in soil. – Journal of Nanoparticle Research 14: 1-14.
[49] Thakur, R. K., Prasad, P., Savadi, S., Bhardwaj, S. C., Gangwar, O. P., Kumar, S. (2022): Nanotechnology for Agricultural and Environmental Sustainability. – In: Goel, R., Soni, R., Suyal, D.C., Khan, M. (eds.) Survival Strategies in Cold-Adapted Microorganisms. Springer, Singapore, pp. 413-424.
[50] Wang, M., Gao, L., Dong, S., Sun, Y., Shen, Q., Guo, S. (2017): Role of silicon on plant–pathogen interactions. – Frontiers in Plant Science 8: 701.
[51] Williams, S. (1984): Official Methods of Analysis of the Association of Official Analytical Chemists. – Association of Official Analytical Chemists, Inc., Washington, DC.
[52] Wu, X., Zhang, S., Liu, X., Shang, J., Zhang, A., Zhu, Z., Zha, D. (2020): Chalcone synthase (CHS) family members analysis from eggplant (Solanum melongena L.) in the flavonoid biosynthetic pathway and expression patterns in response to heat stress. – PLoS One 15(4): e0226537.
[53] Yap, Y. K., El-Sherif, F., Habib, E. S., Khattab, S. (2021): Moringa oleifera leaf extract enhanced growth, yield, and silybin content while mitigating salt-induced adverse effects on the growth of Silybum marianum. – Agronomy 11(12): 2500.
[54] Yassen, A., Abdallah, E., Gaballah, M., Zaghloul, S. (2017): Role of silicon dioxide nano fertilizer in mitigating salt stress on growth, yield and chemical composition of cucumber (Cucumis sativus L.). – Int. J. Agric. Res 22: 130-135.
[55] Ye, M., Song, Y., Long, J., Wang, R., Baerson, S. R., Pan, Z., Zhu-Salzman, K., Xie, J., Cai, K., Luo, S., Zeng, R. (2013): Priming of jasmonate-mediated antiherbivore defense responses in rice by silicon. – Proceedings of the National Academy of Sciences 110(38): E3631-E3639.
[56] Zafar, H., Ali, A., Ali, J. S., Haq, I. U., Zia, M. (2016): Effect of ZnO nanoparticles on Brassica nigra seedlings and stem explants: growth dynamics and antioxidative response. – Frontiers in Plant Science 7: 535.
[57] Zholobenko, A. and Modriansky, M. (2014): Silymarin and its constituents in cardiac preconditioning. – Fitoterapia 97: 122-132.

APPENDIX

Table A1. *Physical and chemical properties of the experimental soil*

Characteristic	Value
Texture	Sandy
Sand %	91.51
Silt %	5.74
Clay %	2.75
Saturation %	23
pH (1:2.5)	7.5
Electrical conductivity (EC) (dS/m)	1.2
Organic matter (OM) %	0.05
Total N %	0.014
Available P ppm	3.9
Available K ppm	110

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Table A2. *Chemical characteristics of the water used for irrigation of S. marianum*

pH	Salinity level (dS/m)	Cations (mmol L^{-1})			Anions (mmol L^{-1})				
		CL^{-1}	$SO4^{-2}$	HCO^{-3}	Ca^{+2}	Mg^{+2}	Na^{+}	K^{+}	Si^{+4}
7.08	1.02	4.21	2.46	0.95	2.44	1.08	6.58	0.25	2.01

Table A3. *Sequences of forward and reverse primers for real-time RT-PCR*

Gene	Primer sequences	Amplicon length (bp)	GenBank accession number
Chalcone synthase 1 (*CHS* 1)	CHS1F 5-TCTTGATTCCCTCGTTGGTC-3 CHS1R 5-TCTCAAACAACGGCCTCTCT-3	101	JN182805.1
Chalcone synthase 2 (*CHS* 2)	CHS1F 5-AGGACATTGCGGAAAACAAC-3 CHS1R 5-AACGGCCTCTCTGTCTTCAA-3	184	JN182806.1
Chalcone synthase 3 (*CHS* 3)	CHS1F 5-ACCCACCTCATCTTTTGCAC-3 CHS1R 5-CATCATGAGGCGTTTGATTG-3	105	JN182807.1
NADH dehydrogenase (*NADH*)	ndhchs_L 5-TTCCGCATTTTGGAAATACC-3 ndhchs_R 5-CCCGTCTTGATTGAAAGGAA-3	134	KC589999.1

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