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Research Article

Biochemical responses of *Silybum marianum* seedlings to independent and combined salt and SiO_2 treatments

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Abstract

In recent years, silicon dioxide (SiO_2) particles have drawn increasing attention due to their potential to enhance plant tolerance to various stresses by modulating physiological and biochemical processes. Salinity is an important abiotic stress with a harmful impact on crop growth and productivity. In this context, the aim of this study was to investigate the influence of silicon dioxide (SiO_2) addition on milk thistle (*Silybum marianum* (L.) Gaertn.) under salinity stress. Thus, individual and combined effects of the application of sodium chloride (NaCl, 50 and 100 mM) and SiO_2 particles (0.1 mM) on some physiological and oxidative stress parameters, such as growth variables, photosynthetic pigments and soluble protein contents, the levels of an oxidative stress biomarker (malondialdehyde), and the specific activity of several antioxidant enzymes (superoxide dismutase, catalase and peroxidase) were evaluated. The results revealed that salt stress (100 mM) inhibited plant growth and decreased chlorophyll and carotenoid levels, whereas the added SiO_2 partially mitigated these negative effects. In addition, the 100 mM NaCl treatment significantly increased peroxidise (POD) activity and decreased soluble protein content after 22 days, while no significant differences were found for group subjected to salt stress in the presence of SiO_2. Results suggest that application of SiO_2 on salt-stressed milk thistle may modify the seedling metabolism and increase the plant salt tolerance capacity.

Keywords: assimilatory pigments; MDA; antioxidant enzymes; salt stress; SiO_2 treatment; milk thistle

Introduction

Silybum marianum (L.) Gaertn. (Commonly known as milk thistle) is an herbaceous plant, annual or biennial, cultivated, sometimes subspontaneous, medicinal, beekeeping. Milk thistle is a plant originally native to southern Europe (Ionescu *et al.*, 2017; Morazzoni and Bombardelli, 1995), but now naturalized throughout

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Europe, the entire American continent and Australia (Khan *et al.*, 2014), and today it is found all over the world (Boyd *et al.*, 2022). It belongs to the Asteraceae family and is known for its distinctive prickly leaves and purple flower heads. Although *S. marianum* is prized for its medicinal properties and intentionally cultivated in various parts of the world (e.g. Egypt, Argentina, China and Europe), in other regions is considered an invasive weed, disturbing the native communities, agricultural crops and pasturelands (Khan *et al.*, 2022).

S. marianum is classified as a medicinal plant that is widely used in traditional European medicine, where it is used especially in the treatment of liver diseases (Morazzoni and Bombardelli, 1995) but it also has antimicrobial, anticancer, antidiabetic, hepatoprotective, cardioprotective, neuroprotective effects, provides skin protection, counteracts the toxicity of antibiotics, heavy metals and pesticides (Wang *et al.*, 2020). The main chemical constituents of this plant are flavonic derivatives, phytosterols, fatty oils, mucilages, pectins, etc. The therapeutic properties of the milk thistle are mainly due to Silymarin (constitutes 1.5-3% of the dry weight of the fruit) which, although it is not the majority constituent, it is considered the most active compound consisting of six isomers namely silybin, isosilybin, silycristin, isosilychristin, silydianin, silymonin (Bijak, 2017). In addition, the fruits also contain other flavonoids such as taxifolin, quercetin, dihydrokaempferol, kaempferol, apigenin, naringin, eriodictyol, chrysoeriol, etc. (Azoz *et al.*, 2019; Bijak, 2017).

Nowadays, agricultural productivity is declining due to the negative effects of climate change, global temperature rises and increased environmental stress (Etesami and Noori, 2019). Among the various types of environmental stress (e.g., extreme temperatures, strong winds, drought, soil salinity), salt stress is known to be one of the most devastating (low agricultural productivity, low economic return and soil erosions) causing major reductions in cultivated area and limiting crop productivity and quality (Shahbaz and Ashraf, 2013).

Abiotic and biotic stresses are a major provocation for agricultural production. To manage these stressful conditions, some techniques, including the use of nanoparticles (NPs), are the ways applied to reduce the difficulties induced by these pressures (Rajput *et al.*, 2021). Numerous studies have demonstrated that SiO_2 addition has beneficial effects on plant development, being effective in enhancing plant resistance to adverse environmental conditions (Rajput *et al.*, 2021). For instance, silicon-mediated enhancement in salt tolerance has been observed in tomato (Al-aghabary *et al.,* 2007), cucumber (Yin *et al.*, 2019; Zhu *et al.,* 2004), barley (Liang *et al.*, 2003), rice (Das *et al.,* 2016), as well as on other plant species (Ivani *et al.,* 2018). The response of plants under abiotic stress in the presence of SiO_2 varied with species, stage of development, treatment duration and concentrations. For example, the tomato was more responsive to silicon treatments than spinach and more pronounced positive effects were observed for higher silicon concentration (5mM) (Gunes *et al.,* 2007).

Our previous studies focused on the salinity effect on medicinal plants such as *Calendula officinalis* (Kozminska *et al.*, 2017) or food plants such as *Petroselinum crispum* (Oprică and Vochita, 2014). In addition, some strategies were used in order to amelioration or remove the harmful effects of this type of stress on plants by using the Bragg Peak proton beam (Oprică *et al.*, 2023), proton Beam (Oprică *et al.*, 2020), silicon nanoparticles (Oprică *et al.*, 2021), PGPR rhizobacterial strains (Oprică and Ştefan 2014).

This work aims to evaluate the impact exerted by the presence of SiO_2 on *S. marianum* (L.) Gaertn. seedlings, by singular treatment and combined with 50 mM and 100 mM NaCl, quantified by the level of some biochemical parameters like antioxidant enzymes activities, lipid peroxidation and soluble protein contents, as well as by photosynthetic pigments like chlorophyll a (Chl a), chlorophyll b (Chl b) and total carotenoids (Caro). Also, the paper addressed the possible application of SiO_2 in the growth, development, and improvement of productivity of the milk thistle plant. To our knowledge, there is currently no information available about the possible beneficial effects of SiO_2 addition on milk thistle subjected to salt stress.

Materials and Methods

Growth conditions and treatments

For this study, we used 30 seeds of *S. marianum*, which were obtained from the Agricultural Research and Development Station in Secuieni (Neamţ, Romania). The seeds were selected so that they were identical in size and color. They were sterilized with 10% H_2O_2 for 30 seconds and then washed extensively with distilled water. The study of the tolerance of *S. marianum* seedlings to the single and, respectively, the combined effect of salt stress and SiO_2 was carried out in laboratory conditions, based on a completely randomized experiment, with three repetitions. The particles SiO_2 used in this study were provided from the VF Kuprevich Institute of Experimental Botany of the National Academy of Sciences of Belarus.

The experimental design consisted of the following six groups: Group 1 – Control, Group 2 – 50 mM NaCl, Group 3 – 100 mM NaCl, Group 4 – 0.1 mM SiO_2 particles, Group 5 – 50 mM NaCl and 0.1 mM SiO_2 particles and Group 6 - 100 mM NaCl and 0.1 mM SiO_2 particles. A pre-treatment was performed before planting, the seeds being exposed to SiO_2 and salt stress alone and in combination for 4 hours in the dark, at room temperature. Then, the control and treated seeds were transferred into pots and incubated in a growth chamber at 20 °C with 16 lights / 8 h dark for 22 days and once every two days, 10 mL of sterile distilled water or exposure solution were added. The selection of SiO_2 concentration was based on our previous study (Oprică *et al*., 2021). Further, the pre-treatment of the seeds was used for promoting more uniform and faster germination (Balde *et al*., 2021). In addition, previous studies have shown that employing the pre-treatment approaches can enhance salt tolerance in plants (Shafiq *et al*., 2019; Baz *et al*., 2020).

Determination of the growth parameters and photosynthetic pigment contents of seedlings

To evaluate the impact induced by treatments on plants, the root length (RL) and shoot height (SH) have been studied. The growth parameters after 22 days were measured manually (Figure 1).

The content of assimilatory pigments (chlorophyll *a*, chlorophyll *b* and carotenoid) in the thistle leaves were performed according to Lichtenthaler (1987) method. Thus, 22-day-old plants were homogenized in 80% acetone. The homogenates were centrifuged at 4 °C and 3,000 rpm for 15 minutes. Finally, the volume used for the analysis was 25 ml. The absorbances at 645, 663 and 470 nm were determined, respectively the pigment content was evaluated and expressed in mg/g fresh weight using the following equations:

Chlorophyll *a* (Chl a) = 12.21 × A663 - 2.81 × A645

Chlorophyll *b* (Chl b) = 20.13 × A645- 5.03 × A663

Carotenoids (Caro)= [(100 × A470 - 3.27 × Chl a - 104 × Chl b) / 227]

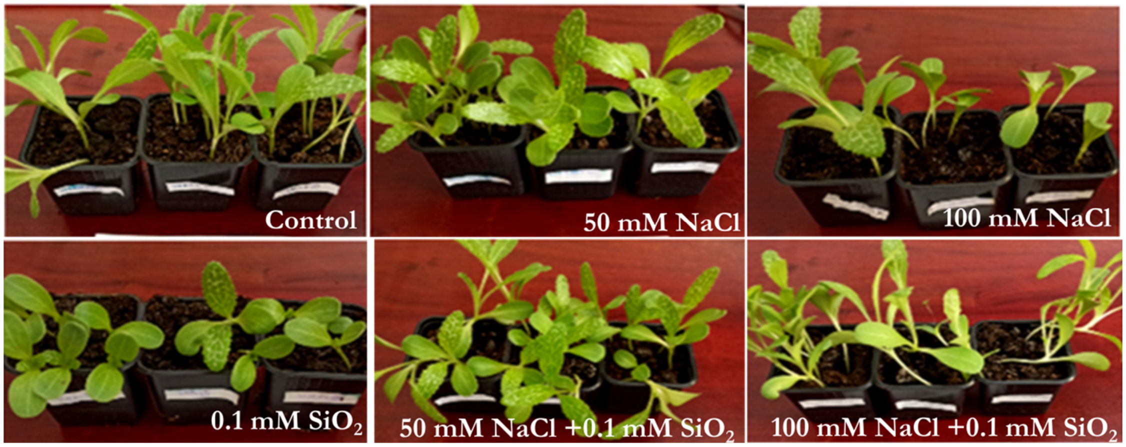

Figure 1. Effect of NaCl and SiO_2 particles on milk thistle growth after 4 h of pre-treatments and 22 days of treatments

Preparation of milk thistle extracts

The preparation of the homogenates was carried out in a manner similar to that previously described by Oprică *et al.* (2015). Thus, the upper parts of *S. marianum* seedlings were weighed individually (~0.5 g), after which they were homogenized with 0.1 M potassium phosphate buffer solution (pH 7.4). After that the homogenate was subjected to cold centrifugation for 15 minutes at 3000 rpm and the supernatant was used to determine the soluble protein content, the specific activity of antioxidant enzymes (SOD, CAT, POD) and the level of MDA.

Determination of superoxide dismutase (SOD) activity

SOD activity was estimated by Winterbourn *et al.* (1975) method, which is based on the ability of SOD to inhibit the reduction of Nitro Blue Tetrazolium (NBT) by the superoxide radicals generated in the reaction mixture by the photoreduction of riboflavin (Oprică *et al.*, 2015). The degree of inhibition produced by the enzyme, under standard conditions, is estimated spectrophotometrically at 560 nm, both in-sample and blank.

Determination of catalase activity (CAT)

CAT activity was measured according to Sinha (1972) method and described in a previous paper (Oprică *et al.*, 2015). The method is based on the fact that potassium dichromate is reduced in acetic acid medium to chromic acetate which in the presence of heat and H_2O_2 and can thus be determined spectrophotometrically at 570 nm. CAT activity was expressed as the amount of enzymes needed to reduce 1μmol of H_2O_2 per min.

Determination of peroxidase activity (POD)

The protocol of peroxidase activity (POD) is based on measuring the color intensity of the oxidized product of ortho-dianisidine with the help of hydrogen peroxide, under the action of POD (Ranieri *et al.*, 2000). One unit of POD activity was expressed as amount of enzymes that produced a change of 1 absorbance per min.

The specific SOD, CAT and POD enzymatic activities were expressed as units per mg protein.

Malondialdehyde (MDA) level determination

The level of MDA was quantified spectrophotometrically according to Hodges *et al.* (1999) and described in a previous paper (Oprică *et al.*, 2015) using of the thiobarbituric acid. Finally, the MDA level was calculated by relating the number of nmol/mg of soluble protein.

Soluble protein content estimation

The number of mg of soluble protein in the enzyme preparations was determined by the Bradford method (Bradford, 1976).

Statistical processing of experimental data

The normality of data distribution was tested with the Shapiro-Wilk test. Afterwards, comparisons among treatments were performed using one-way ANOVA followed by Tukey's test. All statistical analyses were carried out using GraphPad Prism 10.0.2 for Windows (San Diego, CA, USA). The data presented are the means of five replicates ± standard deviation (SD) and for all comparisons, $p \leq 0.05$ was considered significant.

Results

Effect of salinity and SiO_2 treatments on seedlings development of milk thistle seedlings

Results depicted that the 100 mM NaCl treatment has noticeable effects on shoot height (SH) as well as on the content of assimilatory pigments (Chl *a*, Chl b *b* and Caro) in the aerial parts of 22-day-old milk thistle seedlings (Figure 2A-E). Compared with the control, the salt stress (100 mM) decreased the SH by 45%, whereas in the presence of added SiO_2 particles, an only 8% decrease was measured. As can be seen in Figure 2A, no significant differences were found for rooth length between the control and exposed groups. Also, a significant decrease in Chl *a* (by 33%), Chl *b* (by 75%) and Caro (by 17%) was observed in response to 100 mM NaCl as compared to control, while SiO_2 supplementation alleviated the growth inhibition induced by salt stress ($p < 0.05$).

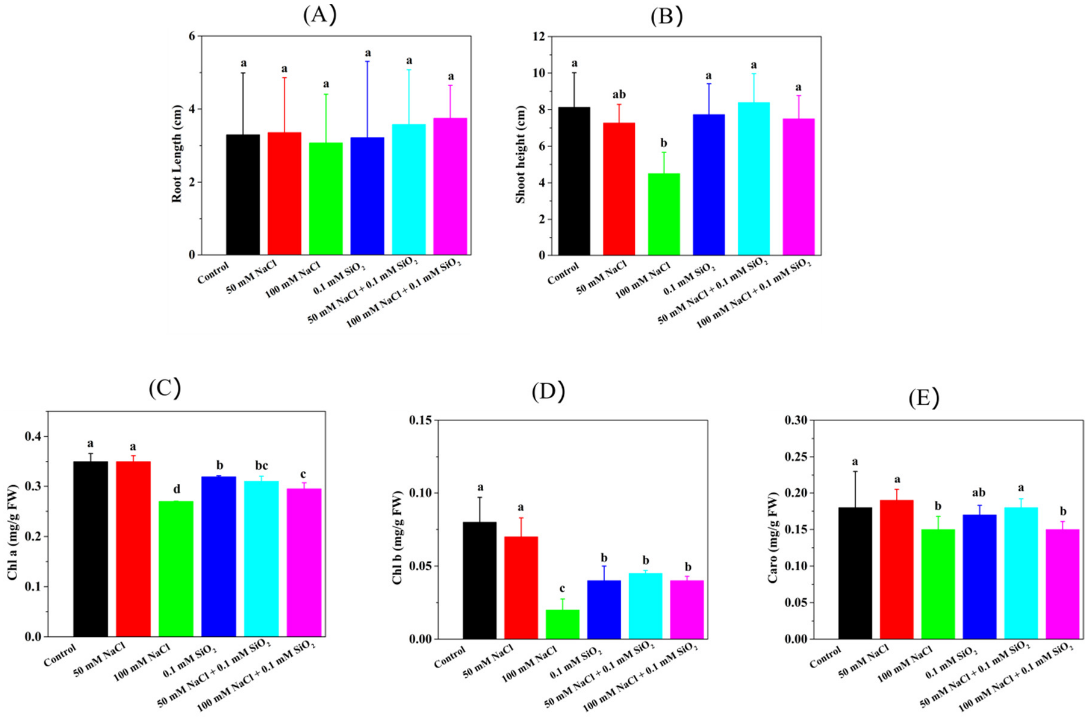

Figure 2. Individual and combined effects of NaCl (50 and 100 mM) and SiO_2 particles application on the (A) root length, (B) shoot height, (C) chlorophyll a, (D) chlorophyll b and (E) carotenes contents in 22-day-old milk thistle seedlings

Data are expressed as mean ± SD (n = 5). Different letters mean significant differences between the groups, according to Tukey's test ($p < 0.05$).

Effect of salinity and SiO_2 treatments on SOD activity of 22-day-old milk thistle seedlings

The results showed that all singular treatments applied to milk thistle seedlings determined a decrease in SOD activity in comparison with control. There is about 18.96%, 17.57% and 19.19% reduction in SOD activity in alone 50 mM, 100 mM and respectively SiO_2 treatments applied to seedlings (Figure 3). On the other hand, the SiO_2 combined treatments with 50 mM and 100 mM NaCl indicated an increase of SOD activity with 14.35% (50 mM + 0.1 mM SiO_2) and 12.24% (100 mM + 0.1 mM SiO_2) (Table 1).

Effect of salinity and SiO_2 treatments on CAT activity of 22-day-old milk thistle seedlings

The data showed that in the variants treated with NaCl (50 mM) there was an increase in CAT activity compared to the control variant by 19.67%, but in the variant that was treated with the concentration of 100 mM NaCl it is noted a decrease, 31.74%, compared to the control variant which could mean that the plant adapts to salt stress, in higher salinity conditions (Figure 3B).

Also, when applying single treatments with SiO_2, CAT activity decreased by 69.84% ($p < 0.001$), which could indicate that SiO_2 does not produce any kind of stress on the plant, because the rate of biosynthesis of antioxidant enzymes was low. Moreover, with the simultaneous application of 50 mM NaCl + 0.1 mM SiO_2, a decrease in CAT activity was observed, compared to the control with 9.50% (Table 1). However, in the case of combined treatment 100 mM NaCl + 0.1 mM SiO_2, CAT activity increased compared to control with 4.75%.

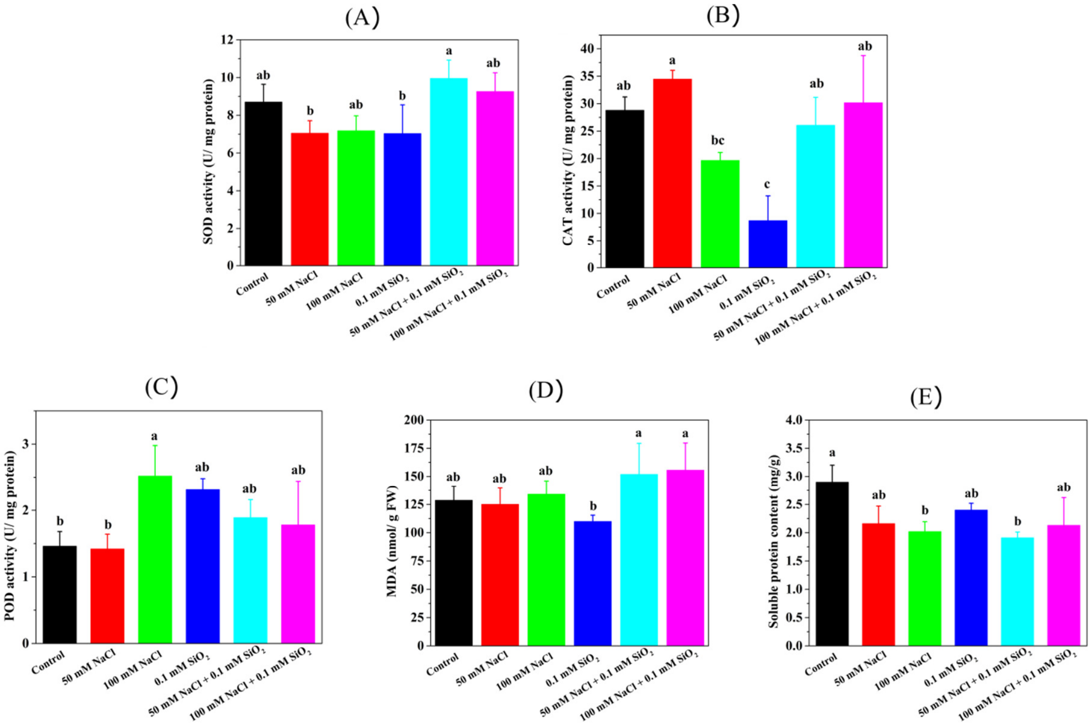

Figure 3. Effect of 0.1 mM SiO2 particles and salt stress (50 and 100 mM) alone and in combination against **(A)** superoxide dismutase (SOD), **(B)** catalase (CAT), **(C)** peroxidase (POD) activities and **(D)** malondialdehyde (MDA) level **(E)** soluble protein content in 22-day-old milk thistle seedlings

Data are expressed as mean ± SD (n = 5). Different letters mean significant differences between the groups, according to Tukey's test ($p < 0.05$).

Table 1. Modulation of the main biochemical indicators after SiO_2 treatment in 22-day-old *S. marianum* seedlings under the conditions as response to salt stress.

Group	SOD activity	± Rate (%)	CAT activity	± Rate (%)	POD activity	± Rate (%)	MDA level	± Rate (%)	Soluble protein content	± Rate (%)
Control	8.70 ± 1.94^{ab}		28.80 ± 2.43^{ab}		1.46 ± 0.22^{b}		128.66 ± 12.5^{ab}		2.90 ± 0.30^{a}	
50 mM NaCl	7.05 ± 0.66^{b}	↓ 18.96	34.47 ± 1.61^{a}	↑ 19.67	1.42 ± 0.22^{b}	↓ 2.73	125.06 ± 14.5^{ab}	↓ 2.47	2.16 ± 0.31^{ab}	↓ 25.77
100 mM NaCl	7.18 ± 0.79^{ab}	↓ 17.57	19.66 ± 1.45^{bc}	↓ 31.74	2.52 ± 0.46^{a}	↑ 72.14	134.01 ± 11.9^{ab}	↑ 9.83	2.02 ± 0.17^{b}	↓ 33.82
0.1 mM SiO_2	7.03 ± 1.52^{b}	↓ 19.19	8.69 ± 4.51^{c}	↓ 69.84	2.31 ± 0.17^{ab}	↑ 58.19	109.89 ± 5.55^{b}	↓ 27.34	2.40 ± 0.12^{ab}	↓ 17.28
50 mM NaCl + 0.1 mM SiO_2	9.95 ± 0.97^{a}	↑ 14.35	26.07 ± 5.09^{ab}	↓ 9.50	1.89 ± 0.27^{ab}	↑ 29.55	151.89 ± 27.5^{a}	↑ 24.66	1.91 ± 0.10^{b}	↓ 34.20
100 mM NaCl + 0.1 mM SiO_2	9.77 ± 4.12^{ab}	↑ 12.24	30.17 ± 8.60^{ab}	↑ 4.75	1.78 ± 0.66^{ab}	↑ 21.99	155.6 ± 24.02^{a}	↑ 26.24	2.13 ± 0.49^{ab}	↓ 26.59

All results are expressed as mean ± SD (n = 5). Different letters mean significant differences between the groups according to Tukey's test.

Effect of salinity and SiO_2 treatments on POD activity of 22-day-old milk thistle seedlings

The obtained results indicate a decrease of 2.73% in POD activity in 22-day-old milk thistle seedlings treated with 50 mM NaCl compared to the control. In the case of the sample treated with 100 mM NaCl ($p < 0.01$), the highest level was reached of POD activity, the increase being 72.14 over the control value, thus indicating a defence reaction of the plant against salinity (Figure 3C).

In the variant treated only with SiO_2, a significant increase in POD activity was also observed, with 58.19%, which indicates the damage to the plant by silica nanoparticles (Table 1). In addition, compared to the control, the combined treatment at the variant with 50 mM + 0.1 mM SiO_2, indicates an increase in POD activity (29.55%), while in the variant with 100 mM + 0.1 mM SiO_2, a small increase in this enzyme was noted with 21.99%.

Effect of salinity and SiO_2 treatments on MDA content of 22-day-old milk thistle seedlings

Saline and SiO_2 treatments applied alone and combined determined a stimulation of MDA content in 22-day-old milk thistle seedlings compared to the plant control (Figure 3D) evidenced that the MDA content in both the 50 mM NaCl treated sample and the control sample is relatively similar, with a decrease of only 2.47% compared to the control. A slight increase, by 9.83%, can be observed in the case of a higher level of NaCl (100 mM). By applying single SiO_2 solutions, a reduction in the MDA level was recorded, by 27.34% (Table 1). On the other hand, the simultaneous application of NaCl + SiO_2, lipid peroxidation measured by the MDA content showed an obvious increase compared to the control and the other variants, by 24.66% (in the case of 50 mM NaCl + 0.1 mM SiO_2) and up to 26.24 % (in the case of the sample treated with 100 mM + 0.1 mM SiO_2), hence resulting that the plants were stressed by NaCl, in combination with SiO_2.

Effect of salinity and SiO_2 treatments on soluble protein contents of 22-day-old milk thistle seedlings

Saline and SiO_2 treatments applied singular and combined determined an inhibition of soluble protein content in 22-day-old milk thistle seedlings compared to the plant control (Figure 3E). Thus, the decrease in soluble protein content was 25.77% and 33.82%, in the case of single treatments of 50 mM NaCl and 100 mM NaCl ($p < 0.01$), respectively (Table 1). The low level of soluble proteins in the case of seedlings subjected to salt stress and treatment with SiO_2, may thus indicate that they were affected by salinity until the time of biochemical determinations. More than that the combined treatment evidenced a decrease with 34.20% in soluble protein content in variants with 50 mM + 0.1 mM SiO_2 ($p < 0.01$) and with 26.59% in those with 100 mM + 0.1 mM SiO_2.

Pearson correlations between enzyme activities and the level of lipid peroxidation

Pearson's correlation coefficient (r) was calculated to identify whether there is a statistically significant correlation between enzyme activity parameters and the level of lipid peroxidation. The results revealed a positive correlation between SOD, CAT and POD activities and MDA level with r of 0.7163 (Figure 4A), 0.6043 (Figure 4B) and 0.3758 (Figure 4C), respectively.

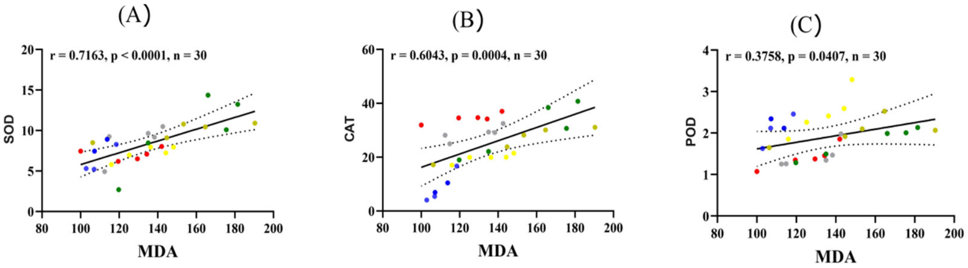

Figure 4. Pearson correlation coefficient between enzyme activities and lipid peroxidation
Linear regression analysis indicated a significant positive correlation between. Values are expressed as means ± SD (n =5 replicates per group).
(A) SOD activity and MDA level (*r* = 0.7163, p < 0.0001);
(B) CAT activity and MDA level (*r* = 0.6043, p = 0.0004);
(C) POD activity and MDA level (*r* = 0.3758, p = 0.0407);
● Ctr, ● 50 mM; ● 100 mM; ● SiO_2; ● 50 mM+ SiO_2; ● 100 mM+ SiO_2.
Data are expressed as follows: SOD (U/mg protein), CAT (μmol H_2O_2 consumed/min/mg protein), POD (U/mg protein), MDA (nmol/g FW).

This can suggest that salt stress has a direct effect on increasing the level of lipid peroxidation assessed by quantifying the MDA content in the leaves of *S. marianum* seedlings. Total MDA content increases significantly with increasing NaCl stress (50 and 100 mM). In contrast, SOD, CAT, and POD activities were activated by salt stress, significantly correlated with MDA level.

Analogously, Zhu *et al.* (2020) showed that in cassava plants under drought stress conditions, there is a positive correlation between the activity of antioxidant enzymes such as SOD, CAT and POD activities and the level of MDA. The authors emphasize the fact that the increase in SOD, CAT and POD activity in the leaves of cassava results in the elimination of superoxide free radicals and in the controlling of MDA levels.

Discussion

Soil salinity is a problem for agricultural productivity worldwide. According to available reports, crops growing in salt affected soils are subjected to osmotic stress, nutritional disturbances and toxicity leading to reduced crop yields (Etesami and Noori, 2019). Soil salinity is one of the major limiting factors that requires special attention due to its detrimental impact on germination, plant growth and development of crop plants (Khalid *et al.*, 2022). Thus, it is necessary to know the reaction environment of plant organisms and to find the physiological, biochemical, and genetic mechanisms that ensure the normal productive potential (Singh *et al.*, 2021).

Salt stress can lead to the development of reactive oxygen species (ROS) in many parts of the plant at the cell level causing a number of changes in a cell's metabolism. To scavenge ROS and cope with oxidative stress, antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and non-enzymatic antioxidant molecules (ascorbate, alkaloids, flavonoids, phenolic compounds, proline, glutathione, α-tocopherol, and carotenoids) are activated (Alam *et al.*, 2022; Khan *et al.*, 2019). The utilization of nutrients may be one of the management approaches for survive with environmental stresses like soil salinity (Kamran *et al.*, 2019). Nanoparticles (NPs) play an important role in plant tolerance to several abiotic stresses and as opposed to fertilizers and pesticides, can improve seed germination, growth and nutrient intake (Alam *et al.*, 2022).

The NPs effect can vary (positive or negative) depending on the size, shape and even the nature of the bulk material from which they are initially synthesized (Behboudi *et al.*, 2018; Khan *et al.*, 2016; Singh *et al.*, 2021). In addition, nanoparticles have also the ability to mitigate the negative effects of various soil stresses such as salinity and drought, which positively influence plant growth morphophysiological traits (Khalid *et al.*, 2022).

Si is needed to ameliorate stress conditions and improve the yields of crops and the exogenous application of this is a recent eco-friendly approach to enhance the salinity stress tolerance in plants (Khan *et al.*, 2019; Mir *et al.*, 2022). Currently, silica nanoparticles (SiO_2) have been described as a new source of Si that can be used more successfully to enhance plant resistance under adverse environmental conditions (Rastogi *et al.*, 2017). In addition, different research has shown the benefits of Si on the plant species development when they are exposed to ecological stresses (Khan *et al.*, 2019). Thus, administration of SiO_2 exogenous treatment reduced salt stress in *Glycine max* by increasing antioxidant activities, K^+ concentration and non-enzymatic components and decreasing lipid peroxidation, generation of reactive oxygen species (ROS) and intracellular Na^+ concentration (Farhangi-Abriz and Torabian, 2018).

Plant growth parameters and photosynthetic pigment contents

The growth of salt-stressed milk thistle seedlings was significantly improved by the addition of SiO_2 particles. For example, shoot height was significantly affected by 100 mM NaCl treatment ($p < 0.01$), but no significant difference was found between the control and the group subjected to salt stress in the presence of SiO_2. This is in line with the findings of Das *et al.* (2016) who showed a decrease in length by about 55% for 100 mM NaCl treatment over water control, while for co-treated seedlings with 100 mM NaCl and 2 mM silicon an average decrease of about only 21% was measured.

Photosynthesis is a process responsible for biomass production and plant development and a variety of stress factors can have a detrimental impact (Rastogi *et al.*, 2019). The degradation of the membranes of thylakoids and chloroplast because of salinity-induced superoxide radicals and H_2O_2 might contribute to the chlorophyll level decrease (Subramanyam *et al.*, 2019). A decrease in these parameters can be associated with the imbalances occurring at the level of cellular functions and with the deterioration of the chain of photosynthetic electron transporters, due to the toxicity of the accumulated ions. The results of this study are in agreement with those obtained by Avestan *et al.* (2019), where the content of assimilatory pigments (chlorophylls *a*, *b* and carotenoids) was significantly reduced in plants subjected to salt stress, compared to controls. More than that, the SiO_2 application partially mitigates the effects of 10 mM NaCl stress by raising chlorophyll a and b contents ($p < 0.05$). These findings are also in agreement with data obtained by González-Moscoso *et al.* (2021), where the presence of the additional SiO_2, led to the improved photochemical efficacy in tomato plants.

Antioxidant defense systems

The overproduction of ROS under salinity stress can be ameliorated by the enzymatic antioxidant plant defence machinery (superoxide dismutase, peroxidase, catalase, ascorbate peroxidase) as well as non-enzymatic antioxidants (ascorbate, flavonoids, carotenoids, stilbenes, tocopherols, and other vitamins). Thus, the oxidative stress can be counteracted either by restoring the level of endogenous antioxidants or by directly detoxifying the overproduced ROS, thereby increasing the plant's tolerance to stress. Increasing antioxidant enzyme levels could reinforce the plant defence system, enabling plants to adapt under different stress conditions (Kesawat *et al.*, 2023).

The present investigation found the negative effect of salinity on SOD activity in 22-day-old milk thistle seedlings, but a significant increase of this enzyme expression in the presence of additional SiO_2, could indicate an improved tolerance efficacy (Figure 3A). A similar finding was found in a study by Singh *et al.* (2021), where the level of SOD activity was higher in the treatment with $NaCl+SiO_2$ particles, than in the case of the control on the *Linum usitatissimum* variants treated with SiO_2 nanoparticles and NaCl (single treatments). Moreover, in the study by Moradbeygi *et al.* (2020) the application of salt stress and nanoparticles with Fe_2O_3, but also the interaction between salinity and nanoparticles, stimulated the *Dracocephalum moldavica* enzymes activities, including SOD.

According to findings (Figure 3B), SiO_2 application induces an increase of CAT activity in milk thistle seedlings in the presence of salt stress in the current investigation. The single application of NaCl in milk thistle seedlings caused an increase in CAT at 50 mM but a decrease at 100 mM. Furthermore, a drop in the enzyme activity was registered upon the application of SiO_2 absence of salt stress. These observations are lined with the study of Nazir *et al.* (2021) who found that CAT activity in *Eriobotrya japonica* seedlings after SiO_2 application was low in the first 3 days but reached peak activity on day 9. Thus, treatment with SiO_2 nanoparticles significantly improved CAT activity after day 9, which contributed to the attenuation of oxidative damage (Wang *et al.*, 2020). Another study (Shekari *et al.*, 2017) also showed that the treatment with Si in salinity conditions increased the SOD, CAT, and APX activities in *Anethum graveolens*. On the other hand, according to research by Zhu *et al.* (2004) on *Cucurbita pepo* L., SiO_2 increased CAT activity.

In the current study (Figures 3C-D), SiO_2 increased the activity of POD and the quantity of MDA in the 22-day-old milk thistle seedlings in conditions of salinity stress (50 and 100 mM), so by the combined application. Wang *et al.* (2011) evidenced that silicon applied to alfalfa under salinity condition increase antioxidative enzyme activity (SOD, POD) and in this way counteract salinity damages. More than that, it could be assumed that SiO_2 particles help the tolerance of the plant, by ameliorating salt stress which can even become harmful.

Soluble protein contents

The continuous generation of ROS in the case of plants subjected to different types of stress causes the occurrence of oxidative stress and, consequently, induces the triggering of various structural, physiological and biochemical disorders such as the decrease in the amount of proteins in the cells (Ayala-Astorga and Alcaraz-Meléndez, 2010; Krishnamoorthy *et al.*, 2022).

SiO_2 and salinity had a negative impact on the production of soluble protein content in 22-day-old milk thistle seedlings (Figure 3E). A characteristic of salt stress is the removal of potassium ions by plant roots, leading to a physiological imbalance, as potassium is required for protein synthesis (Ayala-Astorga and Alcaraz-Meléndez, 2010).

Conclusions

Our findings indicated that salt stress (50 mM and 100 mM), singular or together applied with SiO_2, intensifies the antioxidant defence system in 22-day-old *S. marianum* seedlings, compared to the control. This study suggests that SiO_2 addition in combination with salinity (50 and 100 mM NaCl) can decrease the adverse effects of this kind of stress by regulating the antioxidant defense system. On the other hand, in all treated variants, the soluble protein content in milk thistle seedlings was affected by the single or combined effect of salt stress, in the presence or absence of SiO_2. The total amount of assimilatory pigments (chlorophyll *a*, chlorophyll *b* and carotenes) from the milk thistle seedlings leaves, showed a significant decrease under the action of the 100 mM NaCl treatment, whereas the co-application of SiO_2 led to a better growth of plants. In the present investigation, the application SiO_2 under salt stress enhanced the enzymatic antioxidant machinery for ROS scavenging and mitigating stress. Further studies are necessary to manifest the exact mechanism by which SiO_2 mitigates salinity stress in milk thistle seedlings.

Authors' Contributions

Conceptualization, L.O., G.V., M.-N.G., and L-F.P.; methodology, L.O. and M.-N.G.; investigation, L.O., G.V. and M.-N.G.; writing—original draft preparation, L.O., G.V. and M.-N.G.; statistical processing L.-F. P. and L.O.; project administration and funding acquisition, L.O., G.V. and M.-N.G. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Al-aghabary K, Zhu Z, Shi Q (2007). Influence of silicon supply on chlorophyll content, chlorophyll fluorescence, and antioxidative enzyme activities in tomato plants under salt stress. Journal of Plant Nutrition 27(12):2101-2115. *https://doi.org/10.1081/PLN-200034641*

Alam P, Arshad M, Al-Kheraif AA, Azzam MA, Al-Balawi T (2022). Silicon nanoparticle-induced regulation of carbohydrate metabolism, photosynthesis, and ros homeostasis in *Solanum lycopersicum* subjected to salinity stress. ACS Omega 7(36):31834-31844. *https://doi.org/10.1021/ACSOMEGA.2C02586*

Avestan S, Ghasemnezhad M, Esfahani M, Byrt CS (2019). Application of nano-silicon dioxide improves salt stress tolerance in strawberry plants. Agronomy 9(5):246. *https://doi.org/10.3390/AGRONOMY9050246*

Ayala-Astorga GI, Alcaraz-Meléndez L (2010). Salinity effects on protein content, lipid peroxidation, pigments, and proline in *Paulownia imperialis* (Siebold & Zuccarini) and *Paulownia fortunei* (Seemann & Hemsley) grown *in vitro*. Electronic Journal of Biotechnology 13(5). *https://doi.org/10.2225/VOL13-ISSUE5-FULLTEXT-13*

Azoz SN, Farag HM, Salama AM (2019). Comparative botanical studies two varieties of *Silybum marianum* (L.) Gaertn. (*Asteraceae*) in Egypt. International Journal of Advanced Research in Biological Sciences 6(6):154-168. *https://doi.org/10.22192/ijarbs*

Balde S, Ayessou NC, Sambou A, Niane K, Faye PG, Cisse M, Diop CM (2021). Effects of seed pretreatment on germination, growth and yield of *Memordica charantia* L.. Agricultural Sciences 12(1):39-57. *https://doi.org/10.4236/as.2021.121004*

Baz H, Creech M, Chen J, Gong H, Bradford K, Huo H (2020). Water-soluble carbon nanoparticles improve seed germination and post-germination growth of lettuce under salinity stress. Agronomy 10(8):1192. *https://doi.org/10.3390/agronomy10081192*

Behboudi F, Tahmasebi Sarvestani Z, Kassaee MZ, Modares Sanavi SAM, Sorooshzadeh A (2018). Improving growth and yield of wheat under drought stress via application of SiO_2 nanoparticles. Journal of Agricultural Science and Technology 20(7):1479-1492. *http://jast.modares.ac.ir/article-23-19814-en.html*

Bijak M (2017). Silybin, a major bioactive component of milk thistle (*Silybum marianum* L. Gaernt.)-Chemistry, bioavailability, and metabolism. Molecules (Basel, Switzerland) 22(11). *https://doi.org/10.3390/MOLECULES22111942*

Boyd RJ, Powney GD, Burns F, Danet A, Duchenne F, Grainger MJ, … Pescott OL (2022). ROBITT: A tool for assessing the risk-of-bias in studies of temporal trends in ecology. Methods in Ecology and Evolution 13(7):1497-1507. *https://doi.org/10.1111/2041-210X.13857*

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72(1-2):248-254. *https://doi.org/10.1016/0003-2697(76)90527-3*

Das P, Seal P, Biswas AK (2016). Regulation of growth, antioxidants and sugar metabolism in rice (*Oryza sativa* L.) seedlings by NaCl and its reversal by silicon. American Journal of Plant Sciences 07(03):623–638. *https://doi.org/10.4236/AJPS.2016.73055*

Etesami H, Noori F (2019). Soil salinity as a challenge for sustainable agriculture and bacterial-mediated alleviation of salinity stress in crop plants. Saline Soil-Based Agriculture by Halotolerant Microorganisms *https://doi.org/10.1007/978-981-13-8335-9_1*

Farhangi-Abriz S, Torabian S (2018). Nano-silicon alters antioxidant activities of soybean seedlings under salt toxicity. Protoplasma 255(3):953-962. *https://doi.org/10.1007/S00709-017-1202-0/FIGURES/3*

González-Moscoso M, Martínez-Villegas NV, Meza-Figueroa D, Rivera-Cruz MC, Cadenas-Pliego G, Juárez-Maldonado A, … Juárez-Maldonado A (2021). SiO_2 nanoparticles improve nutrient uptake in tomato plants developed in the presence of arsenic. Revista Bio Ciencias 8. *https://doi.org/10.15741/REVBIO.08.E1084*

Gunes A, Inal A, Bagci EG, Pilbeam DJ (2007). Silicon-mediated changes of some physiological and enzymatic parameters symptomatic for oxidative stress in spinach and tomato grown in sodic-B toxic soil. Plant and Soil 290(1-2):103-114. *https://doi.org/10.1007/S11104-006-9137-9/TABLES/2*

Hodges DM, DeLong JM, Forney CF, Prange RK (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207(4):604-611. *https://doi.org/10.1007/S004250050524/METRICS*

Ionescu D, Spînu S, Orțan A, Moraru I, Fîntîneru G, Fierăscu RC, … Drugulescu M (2017). Evaluation of biological active compounds found in *Silybi mariani fructus*. AgroLife Scientific Journal 6(1):141-145.

Ivani R, Sanaei Nejad SH, Ghahraman B, Astaraei AR, Feizi H (2018). Role of bulk and nanosized SiO_2 to overcome salt stress during fenugreek germination *(Trigonella foenum- graceum* L.). Plant Signaling & Behavior 13(7). *https://doi.org/10.1080/15592324.2015.1044190*

Khalid MF, Iqbal Khan R, Jawaid MZ, Shafqat W, Hussain S, Ahmed T, … Alina Marc R (2022) Nanoparticles: the plant saviour under abiotic stresses. Nanomaterials 12(21):3915. *https://doi.org/10.3390/nano12213915*

Kamran M, Parveen A, Ahmar S, Malik Z, Hussain S, Chattha MS, … Chen JT (2019). An overview of hazardous impacts of soil salinity in crops, tolerance mechanisms, and amelioration through selenium supplementation. International Journal of Molecular Sciences 21(1):148. *https://doi.org/10.3390/IJMS21010148*

Kesawat MS, Satheesh N, Kherawat BS, Kumar A, Kim HU, Chung SM, Kumar M (2023). Regulation of reactive oxygen species during salt stress in plants and their crosstalk with other signaling molecules—Current perspectives and future directions. Plants 12(4):864. *https://doi.org/10.3390/PLANTS12040864*

Khan F, Zahoor M, Ullah N, Khan SN, Khurram M, Khan S, Ali J (2014). A general introduction to medicinal plants and *silybum marianum*. Life Science Journal 11(9s):471-481.

Khan A, Khan AL, Muneer S, Kim YH, Al-Rawahi A, Al-Harrasi A (2019). Silicon and salinity: crosstalk in crop-mediated stress tolerance mechanisms. Frontiers in Plant Science 10:1429. *https://doi.org/10.3389/FPLS.2019.01429/BIBTEX*

Khan MS, Zaka M, Abbasi BH, Rahman L, Shah A (2016). Seed germination and biochemical profile of *Silybum marianum* exposed to monometallic and bimetallic alloy nanoparticles. IET Nanobiotechnology 10(6):359-366. *https://doi.org/10.1049/IET-NBT.2015.0050*

Khan N, Ullah R, Ali K, Jones DA, Khan MEH (2022). Invasive milk thistle (*Silybum marianum (L.) Gaertn.*) causes habitat homogenization and affects the spatial distribution of vegetation in the semi-arid regions of Northern Pakistan. Agriculture 12(5):687. *https://doi.org/10.3390/agriculture12050687*

Kozminska A, Al Hassan M, Kumar D, Oprica L, Martinelli F, Grigore MN, … Boscaiu M (2017). Characterizing the effects of salt stress in *Calendula officinalis* L. Journal of Applied Botany and Food Quality 90:323-329. *https://doi.org/10.5073/JABFQ.2017.090.040*

Krishnamoorthy R, Choudhury AR, Walitang DI, Anandham R, Senthilkumar M, Sa T (2022). Salt stress tolerance-promoting proteins and metabolites under plant-bacteria-salt stress tripartite interactions. Applied Sciences 12(6):3126. *https://doi.org/10.3390/APP12063126*

Liang Y, Chen Q, Liu Q, Zhang W, Ding R (2003). Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgare* L.). Journal of Plant Physiology 160(10):1157-1164. *https://doi.org/10.1078/0176-1617-01065*

Lichtenthaler HK (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods in Enzymology 148(C):350-382. *https://doi.org/10.1016/0076-6879(87)48036-1*

Mir RA, Bhat BA, Yousuf H, Islam ST, Raza A, Rizvi MA, Charagh S, Albaqami M, Sofi PA, Zargar SM (2022). Multidimensional role of silicon to activate resilient plant growth and to mitigate abiotic stress. Frontiers in Plant Science 13:819658. *https://doi.org/10.3389/FPLS.2022.819658/BIBTEX*

Moradbeygi H, Jamei R, Heidari R, Darvishzadeh R (2020). Fe_2O_3 nanoparticles induced biochemical responses and expression of genes involved in rosmarinic acid biosynthesis pathway in Moldavian balm under salinity stress. Physiologia Plantarum 169(4):555-570. *https://doi.org/10.1111/PPL.13077*

Morazzoni P, Bombardelli E (1995). *Silybum marianum (Carduus marianus)*. Fitoterapia, 66:3-42.

Nazir A, Akbar A, Baghdadi HB, Rehman S, Al-Abbad E, Mahvish Fatima M, Iqbal M, Tamam N, Alwadai N, Abbas M (2021). Zinc oxide nanoparticles fabrication using *Eriobotrya japonica* leaves extract: Photocatalytic performance and antibacterial activity evaluation. Arabian Journal of Chemistry (IF 6):14(8) *https://doi:10.1016/j.arabjc.2021.103251*

Oprică L, Grigore MN, Bara I, Vochita G (2021). Salinity and SiO_2 impact on growth and biochemical responses of basil (*Ocimum basilicum* L.) seedlings. International Conference on e-Health and Bioengineering (EHB) *https://doi.org/10.1109/EHB52898.2021.9657645*

Oprică L, Grigore MN, Caraciuc I, Gherghel D, Mihai CT, Vochita G (2020). Impact of proton beam irradiation on the growth and biochemical indexes of barley (*Hordeum vulgare* L.) seedlings grown under salt stress. Plants (9):1234. *https://doi.org/10.3390/PLANTS9091234*

Oprică L, Grigore MN, Vochita G (2015). Impact of saline stress on growth and biochemical indices of *Calendula officinalis* seedlings. Romanian Biotechnological Letters 20(6):11007-11017.

Oprică L, Vochita G (2014). Biochemical changes in two parsley (*Petroselinum crispum* L.) varieties during saline stress. Iranian Journal of Public Health 43(12):1718. *https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4499097/*

Oprică L, Vochita G, Grigore MN, Shvidkiy S, Molokanov A, Gherghel D, Creanga D (2023). Cytogenetic and biochemical responses of wheat seeds to proton irradiation at the bragg peak. Plants 12(4):842. *https://doi.org/10.3390/PLANTS12040842*

Oprică L, Ştefan M (2014). Evaluation of morphological and biochemical parameters of soybean seedlings induced by saline stress. Romanian Biotechnological Letters 19(4):9615-9624 *https://www.researchgate.net/publication/265597966*

Rajput VD, Minkina T, Feizi M, Kumari A, Khan M, Mandzhieva S, Choudhary R (2021). Effects of silicon and silicon-based nanoparticles on rhizosphere microbiome, plant stress and growth. Biology 10(8):791. *https://doi.org/10.3390/BIOLOGY10080791*

Ranieri A, Petacco F, Castagna A, Soldatini GF (2000). Redox state and peroxidase system in sunflower plants exposed to ozone. Plant Science 159(1):159-167. *https://doi.org/10.1016/S0168-9452(00)00352-6*

Rastogi A, Tripathi DK, Yadav S, Chauhan DK, Živčák M, Ghorbanpour M, Brestic M (2019). Application of silicon nanoparticles in agriculture. Biotech 9(3). *https://doi.org/10.1007/S13205-019-1626-7*

Rastogi A, Zivcak M, Sytar O, Kalaji HM, He X, Mbarki S, Brestic M (2017). Impact of metal and metal oxide nanoparticles on plant: A critical review. Frontiers in Chemistry 5:78. *https://doi.org/10.3389/FCHEM.2017.00078/BIBTEX*

Shafiq F, Iqbal M, Ali M, Ashraf MA (2019). Seed pre-treatment with polyhydroxy fullerene nanoparticles confer salt tolerance in wheat through upregulation of H2O2 neutralizing enzymes and phosphorus uptake. Journal of Soil Science and Plant Nutrition 19:734-742. *https://doi.org/10.1007/s42729-019-00073-4*

Shahbaz M, Ashraf M (2013). Improving salinity tolerance in cereals. Critical Reviews in Plant Sciences 32(4):237-249. *https://doi.org/10.1080/07352689.2013.758544*

Shekari F, Abbasi A, Mustafavi SH (2017). Effect of silicon and selenium on enzymatic changes and productivity of dill in saline condition. Journal of the Saudi Society of Agricultural Sciences 16(4):367-374. *https://doi.org/10.1016/J.JSSAS.2015.11.006*

Singh P, Arif Y, Siddiqui H, Sami F, Zaidi R, Azam A, Hayat S (2021). Nanoparticles enhances the salinity toxicity tolerance in *Linum usitatissimum* L. by modulating the antioxidative enzymes, photosynthetic efficiency, redox status and cellular damage. Ecotoxicology and Environmental Safety 213:112020. *https://doi.org/10.1016/J.ECOENV.2021.112020*

Sinha AK (1972). Calorimetric assay of catalase. Analytical Biochemistry 47:389-394. *https://doi.org/10.1016/0003-2697(72)90132-7*

Subramanyam K, Laing GD, Van Damme EJM. (2019). Sodium selenate treatment using a combination of seed priming and foliar spray alleviates salinity stress in rice. Frontiers in Plant Science 10:407836. *https://doi.org/10.3389/FPLS.2019.00116/BIBTEX*

Wang X, Wei Z, Liu D, Zhao G (2011). Effects of NaCl and silicon on activities of antioxidative enzymes in roots, shoots and leaves of alfalfa. African Journal of Biotechnology 10(4):545-549. *https://doi.org/10.5897/AJB10.1353*

Wang X, Zhang Z, Wu SC (2020). Health benefits of *Silybum marianum*: phytochemistry, pharmacology, and applications. Journal of Agricultural and Food Chemistry 68(42):11644-11664. *https://doi.org/10.1021/ACS.JAFC.0C04791/ASSET/IMAGES/MEDIUM/JF0C04791_0007.GIF*

Winterbourn CC, Hawkins RE, Brian M, Carrell RW (1975). The estimation of red cell superoxide dismutase activity. The Journal of Laboratory and Clinical Medicine 85(2):337-341. *https://doi.org/10.5555/URI:PII:0022214375904394*

Yin J, Jia J, H., Lian Z, Hu Y, Guo J, Huo H, Zhu Y, Gong H (2019). Silicon enhances the salt tolerance of cucumber through increasing polyamine accumulation and decreasing oxidative damage. Ecotoxicology and Environmental Safety 169:8-17. *https://doi.org/10.1016/j.ecoenv.2018.10.105*

Zhu Y, Luo X, Nawaz G, Yin J, Yang J (2020). Physiological and biochemical responses of four cassava cultivars to drought stress. Scientific Reports 10(1). *https://doi.org/10.1038/S41598-020-63809-8*

Zhu Z, Wei G, Li J, Qian Q, Yu J (2004). Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.). Plant Science 167(3):527-533. *https://doi.org/10.1016/J.PLANTSCI.2004.04.020*

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