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Using silicon seed priming technology to increase the ability of the medicinal fenugreek plant (*Trigonella foenum-graecum* L.) to withstand the toxicity of ZnO NPs

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Using silicon seed priming technology to increase the ability of the medicinal fenugreek plant (*Trigonella foenum-graecum* L.) to withstand the toxicity of ZnO NPs

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Recently, seed priming has gained significant attention as a technique to enhance stress tolerance in various crop plants. The current research investigated the alleviative effect of seed priming with silicon (Si, 2 mM Na_2SiO_3) on the early growth and antioxidant activity of fenugreek (*Trigonella foenum-graecum* L.), an aromatic and medicinal plant when exposed to zinc oxide nanoparticles (ZnO NPs) toxicity levels (0, 100, and 200 mg L^{-1}). The findings revealed that ZnO NPs toxicity had a notable detrimental effect on growth-related factors. However, Si priming proved effective in attenuating oxidative stress, promoting growth, and increasing the levels of soluble metabolites (soluble carbohydrates, soluble proteins, and free amino acids) in the presence of ZnO NPs toxicity. Moreover, Si priming significantly enhanced both enzymatic and non-enzymatic plant antioxidants compared to non-primed plants under ZnO NPs stress. Therefore, the results of this study support the use of seed priming in Si which was mostly effective in improving the growth and physiological traits of fenugreek plants by alleviating the detrimental effects of ZnO NPs toxicity.

Keywords: Silicon, ZnONPs, Phenolics, Flavonoids, Growth, Metabolites

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INTRODUCTION

Many nanoparticles have beneficial uses, but it is important to note that some can be toxic to both micro and macro-flora, including plants and our food crops (Shukla *et al.*, 2024; Siddiqi and Husen, 2016; Hatami and Ghorbanpour, 2014; Dimkpa *et al.*, 2011). In recent years, several scientists have conducted studies to understand the impact of nanoparticles on seed germination and plant growth, to promote their use in agricultural applications (Hafez and Khalil, 2024; El-Shazoly, 2019b; Dimkpa *et al*., 2011; Nair *et al*., 2010).

Zinc oxide nanoparticles (nano-ZnO) are extensively used in the nano-industry (Meena *et al.*, 2021). Over a decade ago, the presence of nano-ZnO in soil was estimated to range from 3.1 mg/kg to 31 mg/kg (Boxall *et al.*, 2007). Since then, the production of ZnO nanoparticles has significantly increased from 550 to 33,400 tons, and this upward trend continues (Keller *et al.*, 2013; Stroller and Ochando-Pulido, 2020). As a result, the potential risks associated with the environmental distribution of nano-ZnO are considered high (Rajput *et al.,* 2018). The number of studies exploring nano-ZnO use in agriculture is also growing (Yusefi-Tanha *et al.*, 2020). However, the interaction between nano-ZnO and soil systems presents several drawbacks, including accumulation (Kang *et al.*, 2024), transformation (Azarin *et al.*, 2022), bioavailability and potential phytotoxicity (Ahmed *et al*., 2021).

Germination is a critical stage in the life cycle of plants and plays a significant role in crop production. Therefore, there is an urgent need to improve seed quality, including viability and vigor, not only for successful agricultural crop establishment but also to address diverse environmental stressors (Mansour *et al.*, 2019). Seed priming has been recognized as fulfilling all these criteria for managing abiotic and biotic stress, as it enables plants to defend against stressful agents without negatively impacting plant physiology (Ellouzi *et al*., 2021).

Seed priming can be applied through various methods, including hydro-priming (soaking seeds in water) (Kaya *et al.*, 2006), osmo-priming (treating seeds with osmotic solution like mannitol) (Jisha *et al.*, 2013), halo-priming (soaking seeds in inorganic salts solution) (Nawaz *et al.*, 2011), hormo-priming (applying phytohormones) (Attia *et al.*, 2022; Ibrahim *et al.*, 2022), chemical-priming (utilizing chemical agents like melatonin or H_2O_2) (Demir *et al.*, 2012), and nutrient-priming (treating seeds with macro or micro-nutrient solutions) (Alves *et al.*, 2019). These seed priming treatments have been proven to have a positive impact on the dynamics of germination and plant response to heavy metal toxicity (Posmyk *et al.*, 2008; Hu *et al.*, 2009; Sun *et al.,* 2013). Being generally considered the second most abundant element in the earth's crust, after oxygen (Zhu and Gong 2014), timely silicon (Si) is usually classified under the beneficial element category by the International Plant Nutrition Institute (IPNI) (Ouellette

et al., 2017). In recent years, several studies have highlighted the positive effects of silicon (Si) fertilization in agriculture (Thakral *et al.*, 2024; Liang *et al.*, 2015). Si has been found to play a beneficial role in mitigating biotic stresses (Fauteux *et al.*, 2006; Fallah, 2012) and abiotic stresses (Ellouzi *et al*., 2023; El-Shazoly, 2019a; Shi *et al*., 2016; Zhu and Gong, 2014).

Fenugreek, scientifically known as (*Trigonella foenum-graecum* L.), is an annual leguminous crop belonging to the Fabaceae family. It is considered one of the oldest and most significant aromatic and medicinal plants (AMPs), originally found in India and Northern Africa but now cultivated worldwide (Benayad *et al*., 2014). This crop exhibits a moderate tolerance to salinity, drought, and heavy metals, allowing it to thrive in various climatic regions and marginal lands (Lamsaadi *et al*., 2024; Tunçtürk, 2011). Fenugreek seeds and leaves are renowned for their high concentrations of bioactive compounds, which contribute to their therapeutic effects against a wide range of diseases, including diabetes and cancer (Benayad *et al*., 2014; Alrumaihi *et al*., 2021). In this investigation, our focus revolves around the use of silicon (Si) as a priming agent, considering its numerous beneficial roles in managing environmental constraints, particularly concerning ZnO NPs phytotoxicity. The aim of this study is to assess the impact of Si priming on plant biomass, as well as various physiological and biochemical parameters, in fenugreek plants subjected to ZnO NPs phytotoxicity under natural conditions.

MATERIAL AND METHODS

Zinc oxide nanoparticles (ZnO NPs) suspension preparation

The technique of ball milling was used to prepare ZnO NPs in this study from commercial zinc oxide (density of 5.61 g/cm3, 99.5% purity, 81.37 g/mol), obtained from Chem. lab nv company, Belgium (Othman et al., 2018). The characterization of its nanoparticle size is (37±2.6) nm and sonication is used to ensure dispersion for 30 min to prepare the tested suspension of 200 mg/L ZnO NPs.

Growth media preparation and plantation conditions

Sand culturing was used for this experiment, it was prepared as previously described (El-Shazoly, 2019b). Plastic pots filled with 700 grams of the treated sand. The fenugreek seeds (*Trigonella foenum-graecum* L.) used in the experiment provided by the Agricultural Research Center in Giza, Egypt. Seeds were sterilized in a 10% hydrogen peroxide solution for 20 minutes and washed extensively with distilled water. Following sterilization, the seeds were soaked in a solution containing 2mM Sodium silicate (Si) for 12 hours before being sowed in the soil. For the experiment, 15 seeds were planted in sand treated with varying concentrations of ZnO NPs (0, 100, and 200 mg L^{-1}). The soil was maintained at its full field capacity of 100% with an approximate water content of 11%. The control represents the seeds planted solely with distilled water. After germination, the eight healthiest and most uniform seedlings were selected for further analysis. Each treatment was replicated in six plastic pots to ensure precise data collection.

Growth yield and harvest

At the end of the (30 days) experiment, the shoots were carefully separated from the roots. For future analysis, a fresh portion of the harvested shoots was stored at -80ºC. The roots were rinsed with distilled water. To determine the shoots and roots' fresh weight (FW), they were weighed individually, then placed in an oven to remove all moisture at 70ºC for 72 hours to determine dry weight (DW), and water content was measured.

Preparation of plant extract

Fresh fenugreeks shoot samples were ground with 5 ml buffer solution in a chilled mortar and pestle as described by Padmaja et al., (2011). Buffer solution containing 50 mM Tris HCl (pH 7), 1 mM sodium EDTA and 3 mM $MgCl_2$. The extract was centrifuged at 4°C for 10 min at 5000 rpm. The resultant supernatant was used in the determination of soluble metabolites and antioxidants (enzymatic & non-enzymatic).

Soluble metabolites

Soluble carbohydrates: The soluble carbohydrate content was assessed following the method outlined by Fales (1951). The formation of a blue-green color was recorded at 620 nm, and the soluble carbohydrates were quantified using glucose to establish a calibration curve, expressed as mg g^{-1} FW.

Soluble proteins: The determination of soluble protein content was conducted by the procedure described by Lowry et al. (1951) using the Folin reagent method. Soluble proteins were quantified using bovine serum albumin (BSA) to establish a standard curve, expressed as mg g^{-1} FW.

Free amino acids: The free amino acid content was determined following the method outlined by Moore

and Stein (1948). Free amino acids were quantified using glycine to establish a standard curve, expressed as mg g^{-1} FW.

Non enzymatic antioxidants

Flavonoids: Total flavonoid content was measured following the method outlined by Moreno et al. (2000), with quercetin utilized as the standard. The results were represented as mg g^{-1} FW.

Free phenolics: The assessment of phenolic content was carried out using the Folin-Ciocalteu's phenol reagent method cited by Kofalvi and Nassuth (1995). Phenolics were quantified using gallic acid as the standard curve, expressed as µg g^{-1} FW.

Total antioxidants (DPPH%): The stable free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) was assayed with the method reported by Blois (1958). Reference materials were ascorbic acid and BHT. The formula I= (Abs Control-Abs sample)/Abs control x 100. was used to calculate inhibition percentage (I) as radical scavenging activity.

Reducing power: The estimation of reducing power was performed following the method suggested by Oyaizu (1986) with slight modifications. The data are expressed as mg g-1 FW, using ascorbic acid as the standard.

Enzymatic antioxidants

Catalase (EC 1.11.1.6): A spectrophotometric determination was used to determine catalase (CAT) activity following the decrease in H_2O_2 absorbance at A_{240} nm, using the method reported by (Aebi,1984).

Superoxide dismutase (EC 1.15.1.1): The activity of Superoxide dismutase (SOD) was recorded using the adenochrome (autoxidation of epinephrine) as described by (Misra and Fridovich, 1972). The activity was presented as (UE mg^{-1} protein).

Ascorbate peroxidase (EC 1.11.1.11): The ascorbate peroxidase enzyme (APX) activity was assayed by detecting the decrease in absorbance due to the oxidation of ascorbic acid at A290 nm, as cited by Jiang and Zhang. (2002).

Peroxidase (EC 1.11.1.7): The activities of the Peroxidase enzyme (POD) were spectrophotometrically determined by following the absorbance increase at A470 nm, as cited by Tatiana et al. (1999).

Statistical analysis

The experiments were simple, following the one-way Analysis of Variance (ANOVA), and performed under the complete randomized (CR) design. The data was collected from two independent experiments as six measurements in three replicates. The SPSS statistical 11.0 package was utilized to perform the ANOVA analysis. Duncan's as a posthoc test, under $p < 0.05$ as a significance level was conducted as multiple range tests. Principal Component Analysis (PCA) variance regression ordination was employed to analyze the assessed parameters. The circular heatmap was generated using the ggplot packages, R software (RStudio).

RESULTS

Growth yield

Results in Figure 1a, b represents the effects of Si priming on shoot and root fresh weight of fenugreek (*Trigonella foenum-graecum* L.) plants grown under zinc oxide nanoparticle levels (0, 100, and 200 mg L^{-1}). The data showed a significant reduction in shoot and root fresh weight affected by ZnO NPs. On the other hand, significant enhancement was detected in fresh matter gain in shoots treated with Si priming at 100 and 200 mg L^{-1} ZnO NPs by about 75% and 64% enhancement, respectively, when compared to the corresponding treatment without Si (Figure 1). Application of silicon-induced root fresh matter by about 120% and 80%, respectively, under 100 and 200 mg L^{-1} ZnO NPs when compared to corresponding treatments without Si priming. The reduced dry weight of fenugreek plants (shoot and root) because of ZnO NPs applications without Si priming (Figure 2a, b) could be observed. Application of 100 and 200 mg L^{-1} ZnO NPs to the growth media reduced shoot dry matter by 20% and 32%, respectively, while root dry matter reduced by 16% and 33%, respectively, when compared with control. Plants grown from seeds soaked in 2 mM sodium silicate showed significant enhancement in shoot dry weight under 100 and 200 mg L^{-1} ZnO NPs by about 72% and 85%, respectively, when compared to corresponding treatments without Si priming. Data on root dry weight showed improvement but was not significant.

The water content of shoots was reduced significantly due to 100 and 200 mg L^{-1} ZnO NPs applications (Figure 3a). However, the water content of roots (Figure 3b), was reduced significantly at fenugreek plants exposed to the 100 mg L^{-1} ZnO NPs phytotoxicity, but significant induction in root water content %, up to the absolute control level, was observed at 200 mg L^{-1} ZnO NPs. The effect of external Si priming was more obvious in roots than shoots as

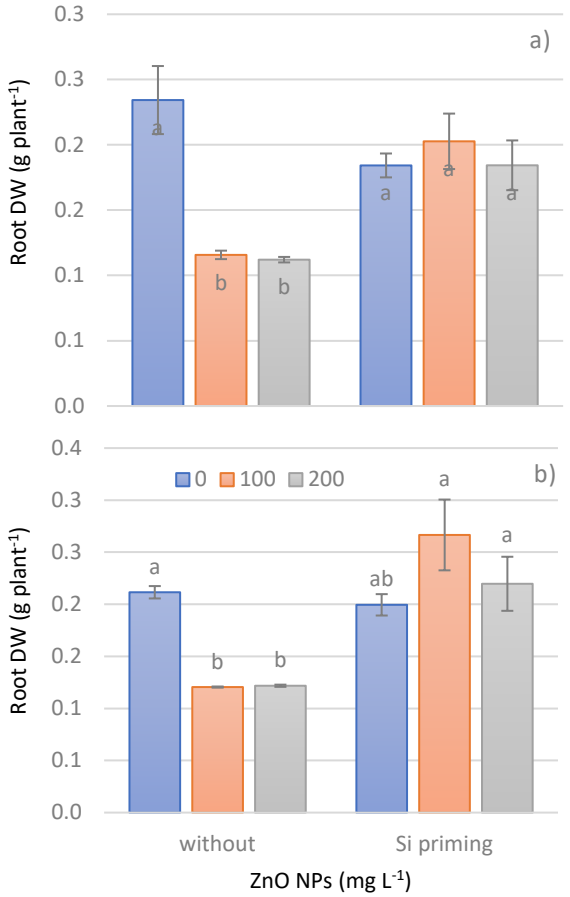

Figure 1. Shoot fresh weight (FW) (1a) and root fresh weight FW (1b) of ZnONPs (0, 100 and 200 mg L^{-1}) treated fenugreek (*Trigonella foenum-graecum* L.) plants as affected by sodium silicate (Si) (as priming agent 2 mM) applications. Values represent means of six replicates, from two independent experiments and the vertical bars indicate ± SE. Different letters above the columns indicate significant difference between treatments, (one-way ANOVA, Duncan's test) at P<0.05 level.

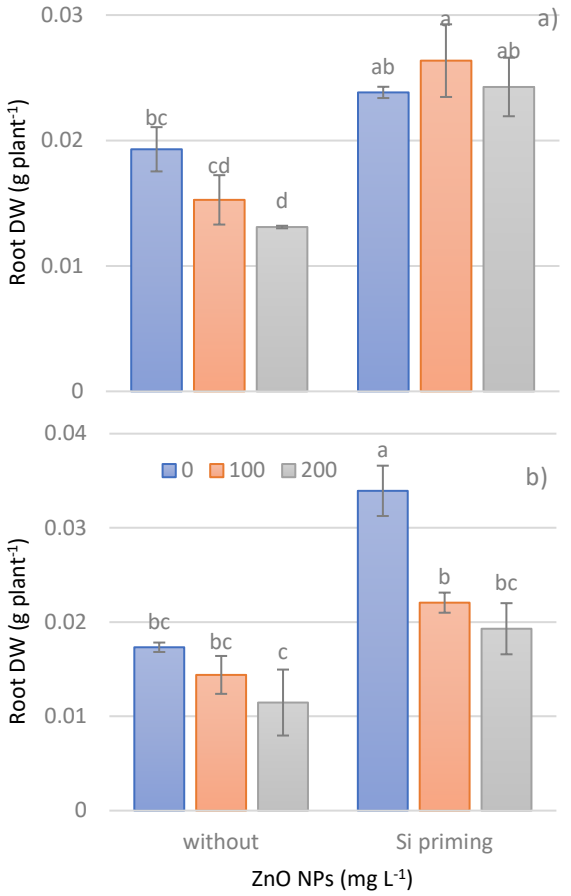

Figure 2. Shoot dry weight (DW) (2a) and root dry weight DW (2b) of ZnONPs (0, 100 and 200 mg L^{-1}) treated fenugreek (*Trigonella foenum-graecum* L.) plants as affected by sodium silicate (Si) (as priming agent 2 mM) applications. Values represent means of six replicates, from two independent experiments and the vertical bars indicate ± SE. Different letters above the columns indicate significant difference between treatments, (one-way ANOVA, Duncan's test) at P<0.05 level.

Si application alleviated root water content % significantly at 100 mg L^{-1} ZnO NPs to the control level, while no such response was observed in shoots (Figure 3a).

Soluble metabolites

The effects of ZnO NPs and Si priming on soluble carbohydrates, soluble proteins and free amino acids, respectively were represented in Figure 4a, b, and c. Data shown in Figure 4a demonstrated that soluble carbohydrate content increased but was not significant in shoots because of ZnO NPs application. While, with the application of Si priming at (0 and 100 mg L^{-1} ZnO NPs), soluble carbohydrates were increased significantly to the highest levels. On the other hand, data on soluble proteins showed an opposite response to soluble carbohydrates; an enhancement in shoot was recorded under 100 mg L^{-1} ZnO NPs level. Increasing the level of ZnO NPs to 200 mg L^{-1} resulted in a significant reduction in soluble protein content. Meanwhile, application of Si-induced soluble protein content in shoot compared to the corresponding treatment under (0 and 100 mg L^{-1} ZnO NPs) (Figure 4b). Also, free amino acid data showed a similar trend to soluble proteins (Figure 4c).

Non enzymatic antioxidants

Flavonoids and free phenolic contents in shoots of fenugreek plants were enhanced with the application of 100 mg L^{-1} ZnO NPs (Figure 5a, b). The content of free phenolics and flavonoids showed no significant change at the higher level of ZnO NPs (200 mg L^{-1}) when compared to the control. On the other hand, a

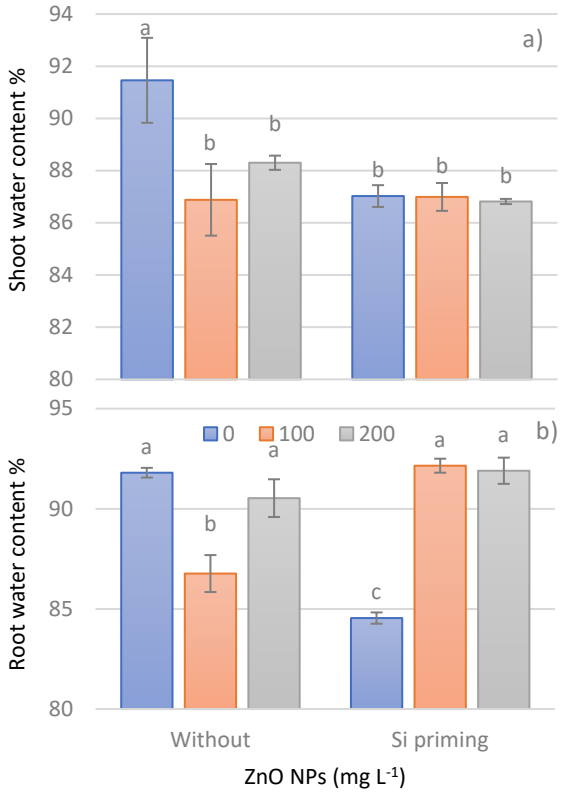

Figure 3. Shoot water content % (3a) and root water content % (3b) of ZnONPs (0, 100 and 200 mg L^{-1}) treated fenugreek (*Trigonella foenum-graecum* L.) plants as affected by sodium silicate (Si) (as priming agent 2 mM) applications. Values represent means of six replicates, from two independent experiments and the vertical bars indicate ± SE. Different letters above the columns indicate significant difference between treatments, (one-way ANOVA, Duncan's test) at P<0.05 level.

significant increase was recorded in free phenolics and flavonoid content in a shoot at 200 mg L^{-1} ZnO NPs level because of Si priming compared to the corresponding treatment without Si priming (Figure 5a, b). Data of total antioxidant DPPH% represented as affected with ZnO NPs phytotoxicity (with or without) Si priming in Figure (6a). ZnO NPs phytotoxicity at 100 mg L^{-1} reduced significantly the total antioxidants% to the lowest level. While the external application of Si as a priming agent increased the content of total antioxidants significantly. On the other hand, increasing the level of ZnO NPs phytotoxicity up to 200 mg L^{-1} with Si priming induced total antioxidant % significantly to the highest level.

Reducing power

Results in Figure 6b exhibit the response of reducing power content as affected by ZnO NPs phytotoxicity (with or without) Si priming. A significant reduction in reducing power content was recorded at 200 mg L^{-1} ZnO NPs phytotoxicity, compared to absolute control.

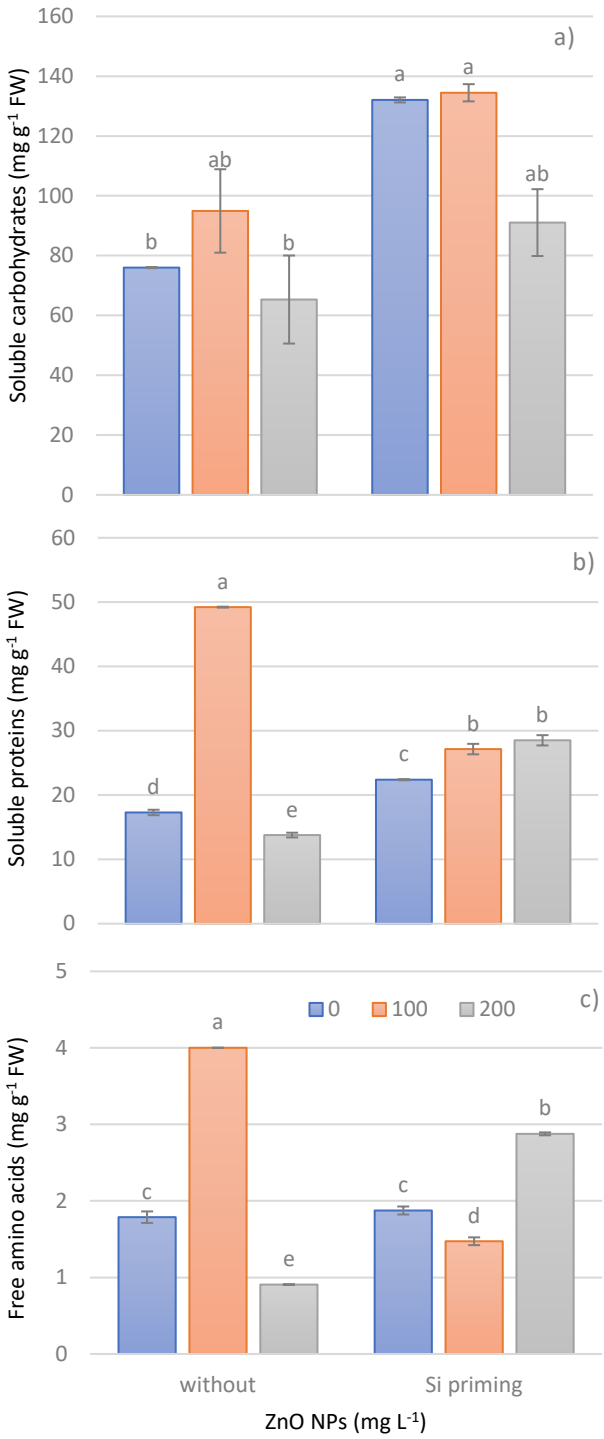

Figure 4. Shoot soluble metabolites; soluble carbohydrates (4a), Soluble proteins (4b) and free amino acids (4c) of ZnONPs (0, 100 and 200 mg L^{-1}) treated fenugreek (*Trigonella foenum-graecum* L.) plants as affected by sodium silicate (Si) (as priming agent 2 mM) applications. Values represent means of six replicates, from two independent experiments and the vertical bars indicate ± SE. Different letters above the columns indicate significant difference between treatments, (one-way ANOVA, Duncan's test) at P<0.05 level.

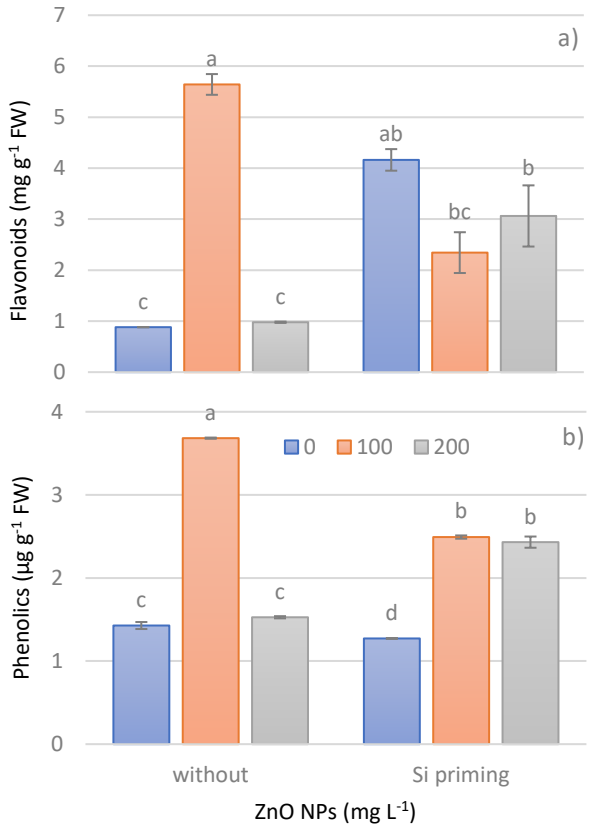

Figure 5. Shoot non-enzymatic antioxidants; flavonoids (5a) and phenolics (5b) of ZnONPs (0, 100 and 200 mg L^{-1}) treated fenugreek (*Trigonella foenum-graecum* L.) plants as affected by sodium silicate (Si) (as priming agent 2 mM) applications. Values represent means of six replicates, from two independent experiments and the vertical bars indicate ± SE. Different letters above the columns indicate significant difference between treatments, (one-way ANOVA, Duncan's test) at P<0.05 level.

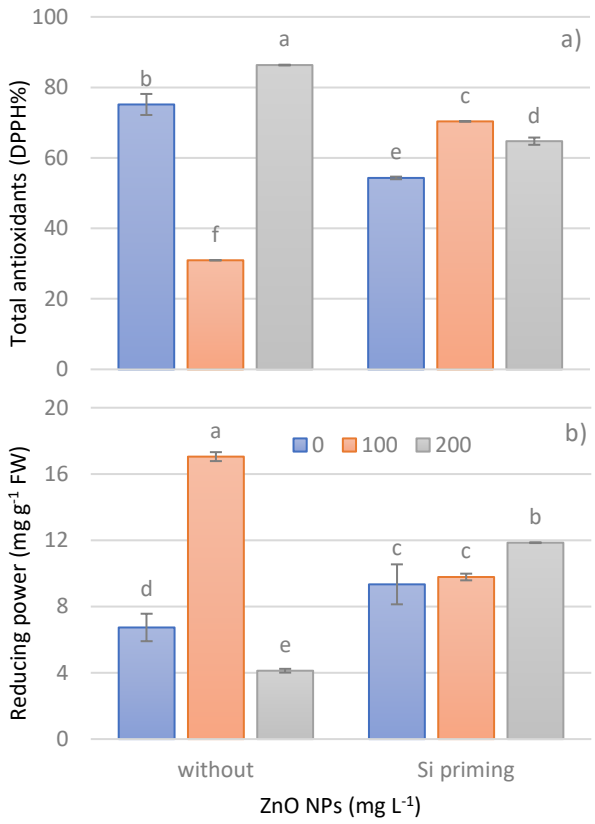

Figure 6. Total antioxidants DPPH % (6a) and reducing power (6b) of ZnONPs (0, 100 and 200 mg L^{-1}) treated fenugreek (*Trigonella foenum-graecum* L.) plants as affected by sodium silicate (Si) (as priming agent 2 mM) applications. Values represent means of six replicates, from two independent experiments and the vertical bars indicate ± SE. Different letters above the columns indicate significant difference between treatments, (one-way ANOVA, Duncan's test) at P<0.05 level.

The external application of Si as priming agent induced reducing power content significantly when compared to 200 mg L^{-1} ZnO NPs level without Si priming. On the other hand, 100 mg L^{-1} ZnO NPs without Si priming significantly induced power content to the highest level.

Enzymatic antioxidants

The activity of CAT in a shoot of fenugreek plants grown under ZnO NPs phytotoxicity showed reduction at 100 and 200 mg L^{-1} ZnO NPs levels (Figure 7a). Application of Si-induced CAT enzyme significantly, when compared to the corresponding treatment without priming. The activity of the SOD enzyme in shoots of fenugreek plants was reduced significantly to the lowest level because of 100 mg L^{-1} ZnO NPs applications (Figure 7b). External application of Si induced SOD activity significantly at 100 mg L^{-1} ZnO NPs level when compared to the corresponding treatment without Si priming. Increasing ZnO NPs concentration in the growth media up to 200 mg L^{-1} increased SOD activity significantly to the highest level. Results in Figures 7c, d demonstrated the activities of ascorbate peroxidase (APX) and peroxidase (POD) enzymes in the shoots of the fenugreek plant as affected by ZnO NPs levels and Si seed priming. APX activity increased significantly due to ZnO NPs application at 100 mg L^{-1}. without Si priming (Figure 7c). On the other hand, POD activity showed no significant change under 100 and 200 mg L^{-1} ZnO NPs application (Figure 7d). Meanwhile, the activities of ascorbate peroxidase and peroxidase enzymes reached their peak because of Si priming at 200 mg L^{-1} ZnO NPs level.

Principal component analysis (PCA)

A principal component analysis (PCA) was conducted on the dataset from the current experiment to find

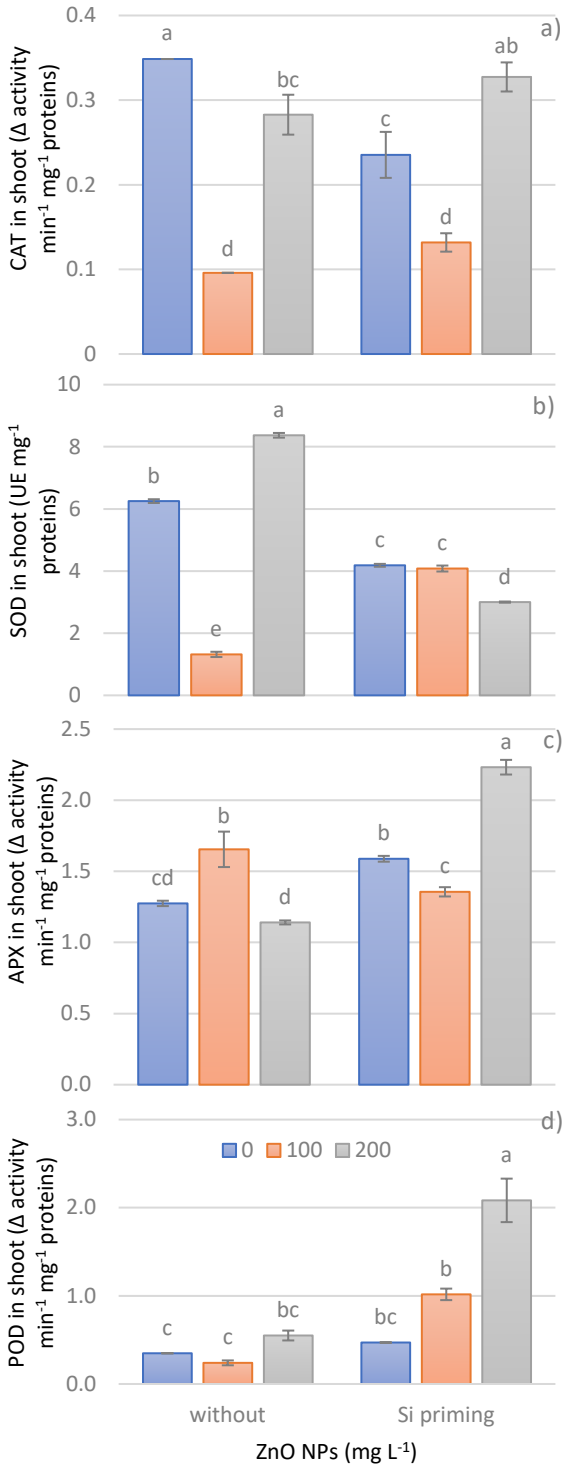

Figure 7. Shoot enzymes; catalase CAT (7a), superoxide dismutase SOD (7b), ascorbate peroxidase APX (7c) and peroxidase POD (7d) of ZnONPs (0, 100 and 200 mg L^{-1}) treated fenugreek (*Trigonella foenum-graecum* L.) plants as affected by sodium silicate (Si) (as priming agent 2 mM) applications. Values represent means of six replicates, from two independent experiments and the vertical bars indicate ± SE. Different letters above the columns indicate significant difference between treatments, (one-way ANOVA, Duncan's test) at P<0.05 level.

the relationships among treatments under different levels of zinc oxide nanoparticles (with or without Si priming). The dataset consisted of 6 treatments and 16 parameters. The biplot provides the positive and negative correlations among the 16 parameters. The closer the parameters are to each other on the biplot, the more similar their trends are. In the right-hand half of Figure 8, there was a noticeable contrast between non-enzymatic antioxidants (phenolics, flavonoids, and reducing power), and soluble metabolites (proteins and amino acids) from this side and parameters in the left-hand half (shoot and root water content%), total antioxidants (DPPH%), and antioxidant enzymes (CAT&SOD). The first axis of the PCA1 captured approximately 43.6% of the cumulative variance, while the second axis of the PCA2 captured 21.1%. The left-hand half was largely influenced by the control treatment and 200 mg L^{-1} ZnO NPs without Si priming (Figure 8). Meanwhile, the right-hand half was greatly influenced by treatments involving 100 mg L^{-1} ZnO NPs without Si priming. It was observed that the lower half collected a positive correlation among growth parameters (fresh and dry weight in shoot and root), carbohydrates, APX, and POD. On the other hand, silicon priming treatments under different ZnO NPs levels (0, 100, and 200 mg L^{-1}) clustered in the lower half of the biplot.

Hierarchical clustering pattern (circular heatmap)

A dendrogram resulted from cluster analysis (Figure 9) with different sub-clusters for all the evaluated attributes and studied treatments. Shoot water content%, root water content%, Total antioxidants DPPH%, and soluble carbohydrates traits showed positive linkages with most treatments, which formed distinct clusters and became separated from other parameters. The remaining parameters such as dry weight (shoot & root), fresh weight (shoot & root), POD, and CAT formed different sub-clusters, showing a negative relationship for most of the treatments.

DISCUSSION

Based on the findings of this study, it was observed that the treatment of fenugreek plants with ZnO NPs resulted in a decrease in the fresh and dry weight of both roots and shoots. However, the presence of Si demonstrated an effect in limiting the decrease in shoot biomass to a greater extent compared to root biomass. Zn tends to accumulate in root vacuoles, where it binds to organic acids. Excessive levels of Zn can lead to reduced biomass, potentially due to the inhibition of meristematic mitoses and the impaired

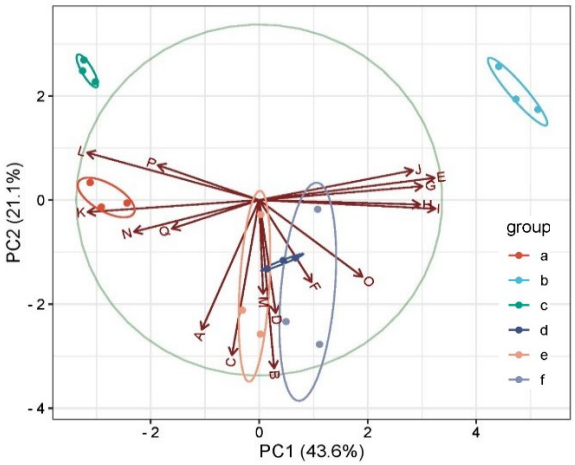

Figure 8. The loading plot of different studied attributes under ZnO NPs treatments correlations to the first two Principal Component analysis (PCA) axes, Horizontal and vertical arrows indicate the rise-direction of ZnO NPs and Si priming treatments. a=control, b=100 mg L^{-1} ZnO NPs, c=200 mg L^{-1} ZnONPs, d=0 mg L^{-1} ZnO NPs +Si priming, e=100 mg L^{-1} ZnO NPs +Si priming, f=200 mg L^{-1} ZnO NPs +Si priming. Parameters: A= shoot fresh weight, B= shoot dry weight, C= root fresh weight, D= root dry weight, E=proteins, F= carbohydrates, G=amino acids, H=flavonoids, I=reducing power, J=phenolics, K=total antioxidants DPPH%, L=SOD, M=POD, N=CAT, O= APX, P= shoot water content%, Q=root water content%.

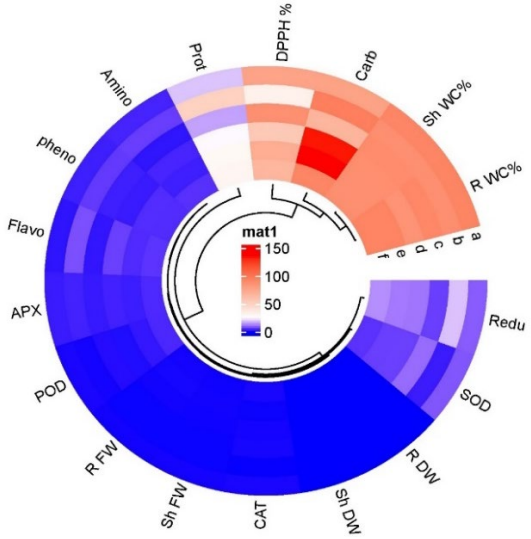

Figure 9. Heatmap graphical display of the relationships among the 6 investigated treatments and 23 measured growth and physiological traits under ZnO NPs levels and GA3 priming application. The different colors and intensities were adjusted based on associations among treatments and traits. The darker blue indicates lower values, while the darker red indicates higher values. Treatments: a=control, b=100 mg L^{-1} ZnO NPs, c=200 mg L^{-1} ZnONPs, d=0 mg L^{-1} ZnO NPs +Si priming, e=100 mg L^{-1} ZnO NPs +Si priming, f=200 mg L^{-1} ZnO NPs +Si priming. Parameters: Sh WC%= shoot water content %, R WC%= root water content %, ShFW= shoot fresh weight, Sh DW= shoot dry weight, RFW= root fresh weight, RDW= root dry weight, Carb=carbohydrates, Amino=amino acids, prot=proteins, Pheno=phenolics, Redu=reducing power, Flavo=flavonoids, DPPH%=total antioxidants.

elongation of root cells. Zn also tends to bind to components of the cell wall, such as structural proteins, cellulose, hemicellulose, and pectin, which disrupts the normal development of roots (Krzesłowska, 2011).

According to Rao and Shekhawat (2014), it was observed that a significant decline in fresh and dry weight occurred as the concentration of ZnO NPs increased in the growth medium. Consistent with the results of this study, Song *et al*. (2011) reported that Si played a significant role in mitigating the reduction in shoot and root biomass caused by excess Zn in two rice cultivars, with a greater impact on shoots than roots. Additionally, Anwaar et al. (2015) found that the addition of 25 and 50 µM Zn to the nutrient solution resulted in a significant decrease in the weight of stems, leaves, and roots in young cotton plants. However, the addition of Si enhanced the plants' ability to cope with Zn excess and reduced the decrease in biomass. Similar findings regarding the ameliorating effect of Si on Zn toxicity have been reported in previous studies (Zajaczkowska *et al.*, 2020; Gu *et al.*, 2012; Kaya *et al.*, 2009). Silicon supply was proven to reduce the translocation of Zn from roots to shoots (Song *et al.*, 2011). Also, Si supply induces the formation of poly-silicate in the cell wall, reinforcing the cell walls and changing the binding capacity for metals, particularly Zn (Brasser *et al.*, 2006). Additionally, Currie and Perry (2007) reported that the integration of silicate compounds in the cell walls of roots increases the number of binding sites for Zn ions, thus enhancing the amount of Zn in the matrix of the cell wall.

The result of water content % indicates a significant reduction in shoot water content% and no significant change in root water content % at 200 mg L^{-1} ZnO NPs. However, a significant reduction in root water content % was recorded at 100 mg L^{-1} ZnO NPs. The effect of silicon application as a priming agent was observed only in root water content at 100 mg L^{-1} ZnO NP, as its application restored root water content significantly up to the control level. Zinc toxicity in plants can have a profound effect on their water content and overall growth. Increased zinc levels in soil and water can hinder the absorption of essential nutrients and disrupt various physiological functions. Previous studies have shown that elevated zinc concentrations can lead to oxidative stress, damaging cellular structures and hindering the plant's ability to maintain turgor pressure, which ultimately results in wilting and decreased water retention (Zhang *et al.*, 2018; Shah *et al.*, 2020). Additionally, zinc toxicity has

been found to negatively affect root development, restricting the plant's access to water and nutrients, thereby worsening water deficit conditions (Sajjad *et al.*, 2019). In species such as *Senna multijuga* and *Erythrina crista-galli*, high soil zinc concentrations have been linked to a linear reduction in the root-specific surface area, negatively impacting growth and diminishing their capacity for water and nutrient absorption (Scheid *et al.*, 2017).

Silicon plays an essential role in supporting plant growth and preserving water content. Studies have indicated that applying silicon can boost a plant's resistance to drought by enhancing water retention and lowering transpiration rates (Gao *et al.*, 2020). Additionally, silicon helps maintain the structural integrity of plant cells by reinforcing cell walls and supporting turgor pressure, which is vital for optimal growth (Baker *et al.*, 2021). Moreover, silicon has been shown to promote root development, enabling plants to access water and nutrients more efficiently from the soil (Kumar *et al.*, 2018). This strengthened root system not only facilitates water absorption but also increases the plant's overall resilience to environmental stressors (Bañuelos *et al.*, 2019).

Soluble metabolites were generally induced at a concentration of 100 mg L^{-1} ZnO NPs, while a significant reduction was observed in soluble carbohydrates, soluble proteins, and free amino acids at a concentration of 200 mg L^{-1} ZnO NPs. However, the external application of silicon significantly increased the levels of soluble metabolites, even at a concentration of 200 mg L^{-1} ZnO NPs. Previous studies have highlighted the protein-rich nature of fenugreek seeds (Nagulapalli *et al*., 2017).

Data inferred from this study indicates that ZnO NPs at a concentration of 200 mg L^{-1} negatively affect the nutritional quality of plants, specifically in terms of soluble metabolites (Fig. 4a, b, c). In contrast, Si priming alleviated the harmful effects of ZnO NPs phytotoxicity and significantly improved the content of soluble proteins, soluble carbohydrates, and free amino acids. The improvement in soluble metabolites recorded at 100 mg L^{-1} ZnO NPs without silicon priming could be attributed to the crucial role of Zinc as a structural stabilizer for various proteins, including transcription factors and enzymes involved in essential physiological processes such as the synthesis and catabolism of biological molecules, from carbohydrates to proteins, and from nucleic acids to lipids (Figueiredo *et al*., 2012; Marschner, 1995; Pedas *et al*., 2009).

However, an excessive amount of Zn can disrupt nutrient homeostasis, negatively affecting mineral uptake and translocation. This disturbance can lead to deficiencies in essential ions like Mg, Fe, and P, ultimately impacting important metabolic processes such as photosynthesis and transpiration (Ali *et al*., 2013; Abbas *et al*., 2009). Additionally, Zn excess has been found to reduce protein, sucrose, and starch levels, as well as affect flowering and seed development in crops like maize and sugar beet (Singh *et al*., 2005).

Our results align with the hypothesis proposed by Bijanzadeh and Egan (2017), suggesting that the alleviating effect of Si may be linked to its osmoprotective role under stressful conditions. Other studies, such as Avila *et al*. (2021) and Hussain *et al*. (2021), have reported the positive influence of Si on plant response to water deficit and shading, respectively, through mechanisms involving osmoregulation and improved carbon metabolism and photosynthesis capability.

The data obtained from the present study indicated that there was no significant change in the content of phenolics and flavonoids when the concentration of ZnO NPs increased to 200 mg L^{-1}. However, a significant increase was observed at 100 mg L^{-1} ZnO NPs. Interestingly, when plants were primed with Si, there was an induction of phenolics and flavonoids even at 200 mg L^{-1} ZnO NPs. Phytotoxicity of ZnO NPs at 200 mg L^{-1} level increased total antioxidants significantly but reduced reducing power content to the lowest value. Application of Si increased reducing power content significantly.

The significant increase, obtained in this study, in the content of phenolics and flavonoids at 100 mg L^{-1} ZnO NPs follows earlier studies that have indicated that plants exposed to ZnO nanoparticles or bulk zinc exhibited heightened levels of flavonoids and phenols, along with increased activity of certain antioxidant enzymes. Some researchers propose that zinc may contribute to the activation of genes involved in the production of antioxidants and secondary metabolic processes (García-López *et al*. 2018; Ma *et al*. 2017). Fenugreek is a valuable medicinal and aromatic plant, owing to its diverse applications in traditional medicine, pharmacy, and cosmetics (Nagulapalli *et al.*, 2017). Fenugreek seeds are particularly known for their high antioxidant and bioactive molecule content, which contributes to their medicinal properties (Alrumaihi *et al.*, 2021). Previous studies have highlighted the richness of

fenugreek seeds in polyphenols, flavonoids, and saponins (Alrumaihi *et al.*, 2021; Nagulapalli *et al.*, 2017; Benayad *et al.*, 2014).

In this study, the data inferred that ZnO NPs at a concentration of 200 mg L^{-1} resulted in a decrease in reducing power content, while no significant change was observed in phenols and flavonoid content. However, Si priming significantly enhanced the content of phenolics and flavonoids and reduced power. Therefore, these findings demonstrate the efficacy of Si seed priming in mitigating the adverse effects of ZnO NPs phytotoxicity on non-enzymatic antioxidants in fenugreek plants.

Reactive oxygen species (ROS) can accumulate in plants experiencing various abiotic and biotic stresses, such as salinity, drought, UV-B radiation, cold, metal toxicity, and pathogen attacks (Das and Roychoudhury, 2014). In response, plants activate their defense systems to eliminate or reduce excess ROS by producing antioxidant enzymes. Among these enzymes, superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) play crucial roles in scavenging excessive ROS, reflecting the dynamic equilibrium of the plant's antioxidant system (Reddy *et al*., 2016).

In this study, the activity of SOD increased significantly at a concentration of 200 mg L^{-1} ZnO NPs, reaching the highest level. SOD acts as the first line of defence against induced damage by ROS, catalyzing the dismutation of superoxide ($O_2^{\cdot -}$) into hydrogen peroxide (H_2O_2) and oxygen (O_2) (Das and Roychoudhury, 2014). While H_2O_2 is beneficial at low concentrations, it can be damaging at higher levels and must be tightly controlled within the cell (Das and Roychoudhury, 2014). POD and CAT, on the other hand, are considered dominant enzymes that convert H_2O_2 into water (H_2O) or other non-toxic products (Kim *et al*., 2012). In this study, Si priming significantly increased the activity of peroxidase (POD), ascorbate peroxidase (APX), and catalase (CAT), reaching the highest level at a concentration of 200 mg L^{-1} ZnO NPs.

Zinc (Zn) serves as a structural stabilizer for numerous proteins and metalloenzymes, including superoxide dismutase (Figueiredo et al., 2012). The activation of antioxidant systems in plants appears to play a crucial role in mitigating metal toxicity (Puppe et al., 2023; Hussain et al., 2015; Sytar et al., 2013; Sharma and Dietz, 2009; Shi et al., 2005). Generally, in the present work, silicon (Si) can alleviate oxidative stress induced by ZnO NPs toxicity by enhancing the activities of enzymatic (e.g., ascorbate peroxidase, peroxidase, and catalase) and non-enzymatic (phenolics and flavonoids) antioxidants. This induction in the antioxidant system could mitigate oxidative stress and help to decrease the accumulation of reactive oxygen species such as hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$), which are known to disrupt cell signaling pathways, leading to severe cell damage or death (Choudhury et al., 2013; Gill and Tuteja, 2010; Kwak et al., 2006). In a meta-analysis, Cooke and Leishman (2016) conducted a statistical assessment of plant responses to Si application under abiotic stress. Their findings indicated that Si consistently alleviates oxidative stress, with variations in responses observed among different plant families.

CONCLUSION

Zinc oxide nanoparticles have a constraining effect on the growth and physiological characteristics of Fenugreek plants. Silicon (Si) has emerged as a promising approach to counteract the phytotoxicity of ZnO NPs. However, there is limited knowledge regarding the impact of Si-seed priming on the growth and physiological responses of fenugreeks under natural conditions in the presence of ZnO NPs toxicity. Si seed priming demonstrated the ability to mitigate Zn toxicity in young fenugreek plants by reducing the decline in biomass. Additionally, silicon enhanced the pool of soluble metabolites (soluble proteins, soluble carbohydrates, and free amino acids) and non-enzymatic antioxidants (phenolics and flavonoids). Furthermore, the beneficial impact of silicon priming extended to the activities of enzymatic antioxidants (CAT, APX, and POD). As a result, the seed priming technique presents a practical, cost-effective, and environmentally friendly approach to crop management. Despite the positive outcomes observed in growth and physiological parameters, further research is warranted to evaluate the effects of these treatments on fungus productivity. This agricultural technique is especially recommended for soils contaminated with ZnO NPs.

REFERENCES

Abbas G, Khan MQ, Jamil M, Tahir M, Hussain F (2009) Nutrient uptake, growth and yield of wheat (*Triticum aestivum*) as affected by zinc application rates. *International Journal of Agriculture and Biology (Pakistan)* 11: 389–396.

Aebi H (1984) Catalase. In Methods in Enzymology (ed. l. paker). Academic press, Orlando, Fl., USA. 105, 121-126.

Ahmed B, Rizvi A, Ali K, Lee J, Zaidi A, Khan MS, Musarrat J (2021) Nanoparticles in the soil–plant system: a review. *Environmental Chemistry Letters* 19: 1545–1609.

Ali S, Farooq MA, Yasmeen T, Hussain S, Arif MS, Abbas F, Zhang G (2013) The influence of silicon on barley growth, photosynthesis and ultra-structure under chromium stress. *Ecotoxicology and environmental safety* 89: 66–72.

Alrumaihi FA, Khan MA, Allemailem KS, Alsahli MA, Almatroudi A, Younus H, Alsuhaibani SA, Algahtani M, Khan A (2021) Methanolic fenugreek seedextract induce sp53-dependentmitotic catastrophe in breast cancer cells, leading to apoptosis. *Journal of Inflammation Research* 14: 1511.

Alves RC, Nicolau CM, Checchio MV, Junior GSS, Oliveira FA, Prado RM, Gratã PL (2019) Salt stress alleviation by seed priming with silicon in lettuce seedlings: an approach based on enhancing antioxidant responses. *Bragantia* 79: 19–29.

Anwaar SA, Ali S, Ali S, Ishaque W, Farid M, Farooq MA, Najeeb U, Abbas F, Sharif M (2015) Silicon (Si) alleviates cotton (*Gossypium hirsutum L*.) from zinc (Zn) toxicity stress by limiting Zn uptake and oxidative damage. *Environmental Science and Pollution Research* 22: 3441–3450.

Attia H, Alamer K, Algethami B, Zorrig W, Hessini K, Gupta K, Gupta B (2022) Gibberellic acid interacts with salt stress on germination, growth and polyamine gene expression in fennel (*Foeniculum vulgare Mill*.) seedlings. *Physiol Mol Biol Plants* 28: 607–622

Avila RG, Magalhães PC, Silva EM, Souza KRD, Campos CN, Alvarenga AA, Souza TC (2021) Application of silicon to irrigated and water defcit sorghum plants increases yield via the regulation of primary, antioxidant, and osmoregulatory metabolism. *agricultural water management.* 255:107004

Azarin K, Usatov A, Minkina T, Plotnikov A, Kasyanova A, Fedorenko A, Alamri S (2022) Effects of ZnO nanoparticles and its bulk form on growth, antioxidant defense system and expression of oxidative stress related genes in *Hordeum vulgare* L. *Chemosphere*, *287*: 132167.

Baker AJM, O'Neill PM, Alford J (2021) The Role of Silicon in Plant Growth and Development. *Journal of Plant Nutrition and Soil Science* 184(5): 597-609.

Bañuelos, G. S., Arroyo, I. M., & Carrillo, J. (2019) Silicon's Role in Enhancing Drought Resistance in Plants: Implications for Agriculture. *Agricultural Water Management*, 213: 181-189.

Benayad Z, Gómez-Cordovés C, Es-Safi NE (2014) Characterization of flavonoid glycosides from fenugreek (*Trigonella foenum-graecum*) crude seeds by HPLC–DAD–ESI/MS analysis. *International Journal of Molecular Sciences* 15: 20668.

Bijanzadeh E, Egan TP (2017) Silicon priming benefits germination, ion balance, and root structure in salt-stressed durum wheat (*Triticum durum* desf.). *Journal of Plant Nutrition* 20: 2560–2571

Blois MS (1958) Antioxidant determinations by the use of a stable free radical. *Nature*. 181(4617): 1199-1200.

Boxall A, Chaudhry Q, Cinclair C, Jones A, Aitken R, Jefferson B, Watts C (2007) Current and Future Predicted Environmental Exposure to Engineered Nanoparticles. Central Science Laboratory, York.

Brasser HJ, Krijger GC, Van Meerten TG, Wolterbeek HT (2006) Influence of silicon on cobalt, zinc, and magnesium in Baker's yeast, *Saccharomyces cerevisiae. Biological Trace Element Research.* 112: 175– 189.

Choudhury S, Panda P, Sahoo L, Panda SK (2013) Reactive oxygen species signaling in plants under abiotic stress. *Plant Signal Behav*., 8: e23681.

Cooke J, Leishman MR (2016) Consistent alleviation of abiotic stress with silicon addition: A meta-analysis. *Functional Ecology* 30, 1340–1357.

Currie HA, Perry CC (2007) Silica in plants: biological, biochemical and chemical studies. *Annals of Botany* 100: 1383–1389.

Das K, Roychoudhury A (2014) Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Frontiers of Environmental Science & Engineering* 2: 1-12

Demir I, Ozuaydın I, Yasar F, Van Staden J (2012) Efect of smoke-derived butenolide priming treatment on pepper and salvia seeds in relation to transplant quality and catalase activity. *South African Journal of Botany* 78:83–87

Dimkpa CO, Calder C, Gajjar P, Merugu S, Huang W, Britt DW, McLean JE, Johnson WP, Anderson AJ (2011) Interaction of silver nanoparticles with an environmentally beneficial bacterium, *Pseudomonas chlororaphis*. Journal of Hazardous Materials, 188: 428-435.

Ellouzi H, Oueslati S, Hessini K, Rabhi M, Abdelly C (2021) Seed priming with H_2O_2 alleviates subsequent salt stress by preventing ROS production and amplifying antioxidant defense in caulifower seeds and seedlings. *Scientia Horticulturae* 288:110–360

Ellouzi H, Rabhi M, Khedher S, Debez A, Abdelly C, Zorrig W. (2023) Silicon seed priming enhances salt tolerance of barley seedlings through early ROS detoxification and stimulation of antioxidant defence. *Silicon*, *15*(1): 37-60.

El-Shazoly RM (2019a) Ameliorating effect of silicon on growth vigor, physiological and biochemical traits of salinized canola seedlings (*Brassica napus* L.). *Catrina journal 18*(1):125-137.

El-Shazoly RM (2019b) Evaluation of the Effectiveness of Safflower (*Carthamus tinctorius*) Seeds Amendment with Silicon or Humic Acid During Germination under CuO (NPs) Phytotoxicity. *EJBO*, *59*(2): 357-374.

Fales FW (1951) The assimilation and degradation of carbohydrates by yeast cells. *Journal of Biological Chemistry*. 193: 113-118.

Fallah A (2012) Silicon effect on lodging parameters of rice plants under hydroponic culture. *International Journal of Agricultural Science* 2:630–634

Fauteux F, Chain F, Belzile F, Menzies JG, Bélanger RR (2006) The protective role of silicon in the Arabidopsis–powdery mildew pathosystem. *Proceedings of the National Academy of Sciences* 103:17554–17559

Figueiredo DD, Barros PM, Cordeiro AM (2012) Seven zinc-finger transcription factors are novel regulators of the stress responsive gene OsDREB1B. *Journal of Experimental Botany* 63: 3643–3656.

Gao Y, Zhang Y, Wang Y (2020) Effects of Silicon on Drought Stress Responses in Plants: A Review. *Frontiers in Plant Science* 11: 1304.

García-López JI, Zavala-García F, Olivares-Sáenz E, Lira-Saldívar RH, Díaz Barriga-Castro E, Ruiz-Torres NA, Ramos-Cortez E, Vázquez-Alvarado R, Niño-Medina G (2018) Zinc oxide nanoparticles boost phenolic compounds and antioxidant activity of *Capsicum annuum* L. during germination. *Agronomy 8* (10):215.

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiology and Biochemistry*., 48: 909–930.

Gu HH, Zhan SS, Wang SZ, Tang YT, Chaney RL, Fang XH (2012) Silicon-mediated amelioration of zinc toxicity in rice (*Oryza sativa* L.) seedlings. *Plant Soil.*, 350: 193–204.

Hafez M, Khalil HF (2024) Nanoparticles in Sustainable Agriculture: Recent Advances, Challenges, and Future Prospects. *Communications in Soil Science and Plant Analysis*, *55*(14): 2181-2196.

Hatami M, Ghorbanpourm M (2014) Defense enzyme activities and biochemical variations of *Pelargonium zonalein* response to nanosilver application and dark storage, *Turkish Journal of Biology,* 38, 130-139.

Hu Y, Ge Y, Zhang C, Ju T, Cheng W (2009) Cadmium toxicity and translocation in rice seedlings are reduced by hydrogen peroxide pretreatment. *Plant Growth Regulation 59*: 51-61.

Hussain S, Mumtaz M, Manzoor S, Shuxian L, Ahmed I (2021) Foliar application of silicon improves growth of soybean by enhancing carbon metabolism under shading conditions. *Plant Physiology and Biochemistry*. 159:43–52.

Hussain I, Ashraf MA, Rasheed R, Asghar, A, Sajid MA, Iqbal M (2015) Exogenous application of silicon at the boot stage decreases accumulation of cadmium in wheat (*Triticum aestivum L.*) grains. *Brazilian Journal of Botany.* 38: 223–234.

Ibrahim MS, Moses N, Ikhajiagbe B (2022) Seed priming with phytohormones. In: Hano CF (ed) Plant hormones - recent advances, new perspectives and applications. IntechOpen, London

Jiang M, Zhang J (2002) Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. *Journal of Experimental Botany* 53: 2401-2410.

Jisha KC, Vijayakumari K, Puthur JT (2013) Seed priming for abiotic stress tolerance: an overview. *Acta Physiologiae Plantarum* 35:1381–1396

Kang M, Liu Y, Weng Y, Wang H, Bai X (2024) A critical review on the toxicity regulation and ecological risks of zinc oxide nanoparticles to plants. *Environmental Science: Nano*.

Kaya C, Tuna AL, Sonmez O, Ince F, Higgs D (2009) Mitigation effects of silicon on maize plants grown at high zinc. *Journal of Plant Nutrition* 32: 1788–1798.

Kaya MD, Okçu G, Atak M, Çıkılı Y, Kolsarıcı Ö (2006) Seed treatments to overcome salt and drought stress during germination in sunfower (*Helianthus annuus* L.). *European Journal of Agronomy* 24: 291–295

Keller AA, McFerran S, Lazareva A, Suh S (2013) Global life cycle releases of engineered nanomaterials. *Journal of Nanoparticle Research* 15: 1692.

Kim S, Lee S, Lee I (2012) Alteration of phytotoxicity and oxidant stress potential by metal oxide nanoparticles in *Cucumis sativus*. *Water Air & Soil Pollut*ion 223: 2799-2806.

Kofalvi SA, Nassuth A (1995) Influence of Wheat Streak mosaic Virus infection phenyl propanoid metabolism and the accumulation of phenolics and lignin in wheat. Physiological and Molecular Plant Pathology 47: 365-377.

Krzesłowska M (2011) The cell wall in plant cell response to trace metals: polysaccharide remodeling and its role in defense strategy. *Acta Physiologiae Plantarum* 33: 35–51

Kumar A, Sharma S, Singh A (2018) Silicon-Induced Growth and Yield Improvements in Plants: A Review. *Agronomy* 8(3): 25.

Kwak JM, Nguyen V, Schroeder JI (2006) The role of reactive oxygen species in hormonal responses. *Plant Physiology*., 141: 323–329.

Lamsaadi N, Ellouzi H, Zorrig W, El Moukhtari A, Abdelly C, Savouré A, arissi M (2024) Enhancing Fenugreek (*Trigonella foenum-graecum* L.) Productivity and Seed Quality through Silicon-Based Seed Priming under Salt-Stressed Conditions. *Russian Journal of Plant Physiology*., *71*(3): 70.

Liang Y, Nikolic M, Belanger R, Gong H, Song A (2015) Silicon in Agriculture: From Theory to Practice. Dordrecht: Springer Netherlands.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin phenol reagent. *Journal of Biological Chemistry.* 193: 265-275.

Ma D, Sun D, Wang C, Ding H, Qin H, Hou J, Huang X, Xie Y, Guo T (2017) Physiological responses and yield of wheat plants in zinc-mediated alleviation of drought stress. *Frontiers in Plant Science* 8:860.

Mansour MM, Ali EF, Salama KH (2019) In: Hasanuzzaman M, Fotopoulos V, Nahar K, Fujita M (eds) Does seed priming play a role in regulating reactive oxygen species under saline conditions? Reactive oxygen, nitrogen and sulfur species. Plants: production,

metabolism, signaling and defense mechanisms, 1st edn. Wiley Online Library

Marschner H (1995) Mineral Nutrition of Higher Plants. 2. Academic Press.

Meena M, Zehra A, Swapnil P, Harish Marwal A, Yadav G, Sonigra P (2021) Endophytic nanotechnology: an approach to study scope and potential applications. *Front. Chem*. 9: 613343.

Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry* 247: 3170-3175.

Moore S, Stein WW (1948) Amino acid free photometric ninhydrin method for use in chromatography of amino acids. *Journal of Biological Chemistry* 176: 367-388.

Moreno MIN, Isla MI, Sampietro AR, Vattuone MA (2000) Comparison of the free radical scavenging activity of propolis from several regions of Argentina. *Journal of Ethnopharmacology* 71: 109–114.

Nagulapalli VKC, Swaroop A, Bagchi D, Bishayee A (2017) A small plant with big benefits: Fenugreek (*Trigonella foenum-graecum Linn*.) for disease prevention and health promotion. *Molecular Nutrition & Food Research* 61, 1600950.

Nair R, Varghese SH, Nair NG, Maekawa T, Yoshida Y, Kumar DS (2010) Nanoparticulate material delivery to plants, *Plant Science*, 179: 154-163.

Nawaz A, Amjad M, Pervez MA, Afzal I (2011) Effect of halopriming on germination and seedling vigor of tomato. *African Journal of. Agricultural Research* 15:3551–3559

Othman AA, Osman MA, Abd-Elrahim AG (2018) The effect of milling time on structural, optical and photoluminescence properties of ZnO nanocrystals. *Optik.* 156: 161-168.

Ouellette S, Goyette M-H, Labbe C, Laur J, Gaudreau L, Gosselin A, Dorais M, Deshmukh RK, Belanger RR (2017) Silicon Transporters and Effects of Silicon Amendments in Strawberry under High Tunnel and Field Conditions. *Frontiers in Plant Science* 8: 949.

Oyaizu M (1986) Studies on product of browning effect reaction prepared from glucose amine. *Journal of Nutrition* 44: 307–315.

Padmaja M, Sravanthi M, Hemalatha KPJ (2011) Evaluation of antioxidant activity of two Indian medicinal plants. *Journal of Phytology*. 3(3).

Pedas P, Schjoerring JK, Husted S (2009) Identification and characterization of zinc-starvation-induced ZIP transporters from barley roots. *Plant Physiology and Biochemistry* 47: 377–383.

Posmyk MM, Kuran H, Marciniak K, Janas KM (2008) Presowing seed treatment with melatonin protects red cabbage seedlings against toxic copper ion concentrations. *Journal of Pineal Research., 45*(1): 24-31.

Puppe D, Kaczorek D, Stein M, Schaller J (2023) Silicon in Plants: Alleviation of Metal(loid) Toxicity and Consequential Perspectives for Phytoremediation. *Plants*, 12: 2407.

Rajput VD, Minkina TM, Behal A, Sushkova SN, Mandzhieva S, Singh R, Gorovtsov A, Tsitsuashvili VS, Purvis WO, Ghazaryan KA, Movsesyan HS (2018) Effects of zinc-oxide nanoparticles on soil, plants, animals and soil organisms: a review. *Environmental nanotechnology, monitoring & manage ment* 9: 76–84.

Rao S, Shekhawat GS (2014) Toxicity of ZnO engineered nanoparticles and evaluation of their effect on growth, metabolism and tissue specific accumulation in *Brassica juncea*. *Journal of Environmental Chemical Engineering.*, *2*(1): 105-114.

Reddy PV, Hernandezviezcas JA, Peraltavidea JR, Gardeatorresdey JL (2016) Lessons learned: are engineered nanomaterials toxic to terrestrial plants? *Science of the Total Environment*. 568: 470-479.

Sajjad Y, Ali A, Baloch MS (2019) The Role of Zinc in Plant Stress Response and Water Relations. *International Journal of Agriculture and Biology*, 21(3): 613-619.

Scheid DL, De Marco R, Grolli AL, Da Silva RF, Da Ros CO, Andreazza R (2017) Growth, tolerance and zinc accumulation in *Senna multijuga* and *Erythrina crista-galli* seedlings. *Revista Brasileira de Engenharia Agrícola e Ambiental* 21:465–470.

Shah AA, Ali S, Khan MI (2020) Impact of Heavy Metal Toxicity on Plant Growth and Water Relations. *Journal of Plant Growth Regulation*, 39(2): 296-308.

Sharma SS, Dietz KJ (2009) The relationship between metal toxicity and cellular redox imbalance. *Trends in Plant Science* 14: 43–50.

Shi X, Zhang C, Wang H, Zhang F (2005) Effect of Si on the distribution of Cd in rice seedlings. *Plant Soil* 272: 53–60.

Shi Y, Zhang Y, Han W, Feng R, Hu Y, Guo J, Gong H (2016) Silicon enhances water stress tolerance by improving root hydraulic conductance in *Solanum lycopersicum* L. *Frontiers in Plant Science* 7:196

Shukla K, Mishra V, Singh J, Varshney V, Verma R, Srivastava S (2024) Nanotechnology in sustainable agriculture: A double-edged sword. *Journal of the Science of Food and Agriculture*, *104*(10): 5675-5688.

Siddiqi KS, Husen A (2016) Fabrication of metal and metal oxide nanoparticles by algae and their toxic effects. *Nanoscale Research Letters* 11: 363.

Singh B, Natesan SKA, Singh BK, Usha K (2005) Improving zinc efficiency of cereals under zinc deficiency. *Current Science India,* 88: 36–44.

Song A, Li P, Li Z, Fan F, Nikolic M, Liang Y (2011) The alleviation of zinc toxicity by silicon is related to zinc transport and antioxidative reactions in rice. *Plant Soil* 344: 319–333.

Stoller M, Ochando-Pulido JM (2020) ZnO Nano-particles production intensification by means of a spinning disk reactor. *Nanomaterials* 10: 1321.

Sun J, Wang R, Zhang X, Yu Y, Zhao R, Li Z, Chen S (2013) Hydrogen sulfide alleviates cadmium toxicity through

regulations of cadmium transport across the plasma and vacuolar membranes in Populus euphratica cells. *Plant Physiology and Biochemistry*, *65*: 67-74.

Sytar O, Kumar A, Latowski D, Kuczynska P, Strzałka K, Prasad MNV (2013) Heavy metal-induced oxidative damage, defense reactions, and detoxification mechanisms in plants. *Acta Physiologiae Plantarum* 35: 985–999.

Tatiana Z, Yamashita K, Matsumoto H (1999) Iron deficiency induced changes in ascorbate content and enzyme activities related to ascorbate metabolism in cucumber roots. *Plant and Cell Physiology*. 40: 273-280.

Thakral V, Raturi G, Sudhakaran S, Mandlik R, Sharma Y, Shivaraj SM, Deshmukh R (2024) Silicon, a quasi-essential element: Availability in soil, fertilizer regime, optimum dosage, and uptake in plants. *Plant Physiology and Biochemistry 208*: 108459.

Tunçtürk R (2011) Salinity exposure modifies nutrient concentrations in fenugreek (*Trigonella foenum graecum L.*). *African Journal of. Agricultural Research* 6 (16): 3685-3690.

Yusefi-Tanha E, Fallah S, Rostamnejadi A, Pokhrel LR (2020) Zinc oxide nanoparticles (ZnONPs) as a novel nanofertilizer: influence on seed yield and antioxidant defense system in soil grown soybean (*Glycine max* cv. Kowsar). *Science of the Total Environment*. 738: 140240.

Zajaczkowska A, Korzeniowska J, Sienkiewicz-Cholewa U (2020) Effect of soil and foliar silicon application on the reduction of zinc toxicity in wheat. *Agriculture*, *10*(11): 522.

Zhang Y, Wang Y, Liu X (2018) Effects of Zinc Toxicity on Growth, Physiological Parameters, and Antioxidant Enzyme Activity in Plants. *Environmental Science and Pollution Research*, 25(18): 17844-17855.

Zhu Y, Gong H (2014) Beneficial effects of silicon on salt and drought tolerance in plants. *Agronomy for Sustainable Development* 34:455–472.