Iron Oxide and Silicon Nanoparticles Modulate Mineral Nutrient Homeostasis and Metabolism in Cadmium-Stressed Phaseolus vulgaris

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The application of nanoparticles (NPs) has been proved as an efficient and promising technique for mitigating a wide range of stressors in plants. The present study elucidates the synergistic effect of iron oxide nanoparticles (IONPs) and silicon nanoparticles (SiNPs) in the attenuation of Cd toxicity in Phaseolus vulgaris. Seeds of P. vulgaris were treated with IONPs (10 mg/L) and SiNPs (20 mg/L). Seedlings of uniform size were transplanted to pots for 40 days. The results demonstrated that nanoparticles (NPs) enhanced growth, net photosynthetic rate, and gas exchange attributes in P. vulgaris plants grown in Cd-contaminated soil. Synergistic application of IONPs and SiNPs raised not only K⁺ content, but also biosynthesis of polyamines (PAs), which alleviated Cd stress in P. vulgaris seedlings. Additionally, NPs decreased malondialdehyde (MDA) content and electrolyte leakage (EL) in P. vulgaris plants exposed to Cd stress. These findings suggest that stress alleviation was mainly attributed to the enhanced accumulation of K⁺ content, improved antioxidant defense system, and higher spermidine (Spd) and putrescine (Put) levels. It is suggested that various forms of NPs can be applied synergistically to minimize heavy metal stress, thus increasing crop production under stressed conditions.

Keywords: growth, potassium, polyamines, antioxidant, stress, cadmium
INTRODUCTION

Mining operation, industrial waste, and pollutants emitted during agricultural and industrial operations (Palansooriya et al., 2020; Raghib et al., 2020) release substantial quantities of toxic heavy metals (As, Cd, Hg, Pb) into the environment. The quantity of harmful heavy metals in the soil environment has steadily increased over time, which produces negative effects on plant growth and their developmental process, which is a great challenge for the sustainability of agriculture (Afzal et al., 2019). Among the numerous heavy metals, Cd content in the environment has risen owing to excessive use of chemical fertilizers and pesticides besides mining sources (Seshadri et al., 2016; Manzoor et al., 2019). Although the concentration of Cd reported in the environment is low, it is considered extremely toxic due to its higher mobility in media and living cells (Rahman and Singh, 2019; Kaya et al., 2020a), which is raising concerns for agriculture. It was reported that Cd exposure reduces seed sprouting, root elongation, shoot development, and the number of leaves per plant (Kaya et al., 2020b; Seifikalhor et al., 2020). In addition, it also results in the overproduction of reactive oxygen species (ROS) (Moradi-Marjaneh et al., 2019), which influence plant physiological, biochemical, and molecular characteristics (Raza et al., 2020). Plants can avoid, tolerate, and immobilize heavy metals in soil (Kumar and Prasad, 2018; Mei et al., 2020) by activating signaling molecules that regulate ROS production, phytohormone synthesis, and calcium calmodulin pathways (Bali et al., 2019). The activated pathways include the production of nitric oxide (NO), glutathione, phytohormones, and antioxidant enzymes (Dias et al., 2019; Rady et al., 2019), which lead to Cd chelation and reduced oxidative damage.

Nanoparticles (NPs) with a size of 100 nm exhibit unique properties such as increased reaction site, high surface activity, better catalytic efficiency, and unique magnetic characteristics (Yang et al., 2017, 2018; Wang et al., 2019), which have assisted in agriculture promotion by alleviating abiotic stress in plants (Adeel et al., 2019). Many researchers have reported that NPs improve seed germination, rhizome development, and quality of crops cultivated in stressed conditions (Ghafariryan et al., 2013; Palchoudhury et al., 2018; Kah et al., 2019). Nanotechnology research offers a new pathway for soil pollution remediation (Liu et al., 2021), since it has the potential to enhance plants’ antioxidative defense systems, thus reducing the bioaccumulation of ROS in plants (Usman et al., 2020; Wang et al., 2020). As documented, NPs when applied in soil (Li et al., 2021) or sprayed via foliar (Toumey, 2020), results in a reduction of Cd and Pd toxicity in rice seedlings (Hussain et al., 2020). It was observed that the Si improved hydraulic conductivity in applied plants through enhancing K+ concentration in xylem sap resulting in improved xylem hydraulic conductivity and osmotic gradient (Chen et al., 2016). Similarly, exogenously applied Fe modulates K+ uptake and translocation in crop plants (Okture Asri and Sonmez, 2014).

Potassium is a crucial phytonutrient required by plants for the maintenance of growth (Jaiswal et al., 2016), stomatal conductance, gaseous exchange (Hasanuzzaman et al., 2018), photosynthate production, and antioxidative defense system (Xu et al., 2020). Approximately 10% of plant biomass is composed of K, which not only acts as a co-factor for various enzymes involved in photosynthesis and protein stabilization, but it also hinders Cd-translocation and escalates the production of crucial amino acids, carbohydrates, and nitrogenous compounds. Potassium improves plant stress tolerance by regulating the biosynthesis of polyamines (Karimi, 2017). Polyamines regulate physiochemical procedures of plants under normal conditions and also induce modification in the expression level of stress-responsive genes besides detoxifying metals by vascular compartmentalization (Spormann et al., 2021).

Considering the background information, we hypothesized that the synergistic effect of NPs can mitigate Cd stress; and regulate polyamine synthesis, K+ metabolism, and antioxidant enzymes. Therefore, the objective of the present study was to explore the individual and combined role of IONPs and SiNPs application in mitigation of Cd toxicity through regulation of K+ metabolism and antioxidant enzymes in P. vulgaris exposed to Cd-contaminated conditions.

MATERIALS AND METHODS

A total of 85 soil samples were collected (0–30 cm) from an agricultural field (2-ha area) in the vicinity of the campus site. The soil was sieved through a 4 mm mesh to remove plant parts, debris, then thoroughly mixed and conditioned for 1 week at 35% of water holding capacity (WHC) before the experiment. The physicochemical properties of the soil such as pH, electrical conductivity (EC), organic matter, and metal content (Cd, Zn, Fe, Ni, Pb) were measured using standard protocols (Bouyoucos, 1962; Page et al., 1982). The physicochemical properties of the soil were as follows; pH (7.13), EC (1.87), and organic content (0.45%). The total concentration of Cd, Zn, Fe, Ni, and Pb in soil was quantified as 5.87, 34.98, 37.91, 13.28, and 35.18 mg/kg, respectively. The soil was contaminated by mixing CdCl2. Cadmium concentrations in the soil were kept as 0 mM CdCl2, 1 mM CdCl2, 1.5 mM CdCl2, and 2 mM CdCl2. Both IONPs and SiNPs were purchased from Alfa Aesar. A pilot project was carried out to find out the toxic concentration of Cd that affects the growth of P. vulgaris. A 10 mg/L IONPs and 20 mg/L SiNPs were found to be effective against selected Cd toxic levels. Iron oxide nanoparticles (IONPs) obtained from Alfa Aesar were having 99% purity, size 15–25 nm, and 4.67 density. Silicon nanoparticles obtained from Alfa Aesar were having 98% purity, size 40–100 nm, and 4.7 density. The solution used during the experiment was prepared using deionized H2O.

Seed Priming With Iron Oxide Nanoparticles and Silicon Nanoparticles

Seeds of P. vulgaris were surface sterilized using 2.5% sodium hypochlorite solution for 2 min and then washed with deionized H2O to remove chlorophyll (Chl) contents. IONPs and SiNPs were weighed and added in deionized H2O followed by ultra-sonication for half-hour and their desired concentrations (IONPs = 10 mg/L) and (SiNPs = 20 mg/L) were achieved. After that sterilized seeds of P. vulgaris were soaked in an NP solution.
In the case of the control treatment, seeds were treated with deionized H₂O. In the next step, soaked seeds were dried and stored at 4°C for further experiments.

**Seed Sowing and Greenhouse Conditions**

The current study was executed at the university site (32°N, 74°E, 236 m altitude). Ten seeds were sown in plastic pots of 40 × 45 cm containing 20 kg treated soil. These pots were kept in the greenhouse condition with an average temperature of 20/15°C (day/night), average humidity of 50/70% late afternoon/morning, and a natural photoperiod. After germination, thinning was done and 8 plants were retained per pot. Regular weeding was done and plants were watered daily to uphold 70% of the field maximum moisture capacity throughout the growth period.

**Determination of Growth and Leaf Relative Water Content**

Plants were uprooted after 40 days and growth characteristics (root and shoot fresh weight, root and shoot dry weight) were estimated. Leaf relative water content (LRWC) values from *P. vulgaris* leaf samples were estimated using the following equation as given by Smart and Bingham (1974);

\[
LRWC(\%) = \frac{LFW - LDW}{LTW - LDW} \times 100
\]

Where LRWC = Leaf relative water content

LFW = Leaf fresh weight

LDW = Leaf dry weight

LTW = Leaf turgid weight

**Estimation of Photosynthetic Pigments**

Arnon method (1949) was used for the estimation of photosynthetic pigments. Approximately 100 mg leaf extract was mixed with 80% acetone and centrifuged for 5 min at 10,000 rpm. The optical density (OD) of filtrates was then measured using a spectrophotometer (Hitachi U-2001, Tokyo, Japan).

**Determination of Malondialdehyde Content and Electrolyte Leakage**

Malondialdehyde (MDA) content was determined according to the method of Cavalcanti et al. (2004). Briefly, 0.5 g samples were homogenized using mortar and pestle in 4 mL of trichloroacetic acid (TCA) (1% w/v) at 4°C. The homogenous mixture was then centrifuged for 20 min at 12,000 rpm to collect the supernatant. One milliliter of this supernatant was added to 3 mL of reaction mixture containing TCA (20% v/v) and thiobarbituric acid (0.5% w/v). The reaction mixture was incubated at 95°C for half-hour and the reaction was stopped by placing it in an ice bath. The absorbance value of the fraction was measured at 440, 532, and 600 nm.

The method of Blum and Ebercon (1981) was used for the determination of electrolyte leakage. A dry leaf sample (0.2 g) was floated in deionized H₂O (50 mL) for 24 h at room temperature with shaking. The electrolyte content in the solution was quantified and recorded as CO. After 20 min of boiling the sample, the electrolyte content in the solution was recorded as C1. Electrolyte leakage was measured in percentage as per the following formula:

Relative electrolyte leakage = \( \frac{CO}{C1 \times 100} \)

**Determination of Antioxidant Enzymes**

About 1 g leaf samples were homogenized in a solution of potassium phosphate buffer solution (100 mM), polyvinyl pyrrolidone (1% w/v), ethylenediamine tetraacetic acid (0.1 mM) and triton X-100 (0.5%). The homogenate was filtered through cheesecloth and centrifuged at 18,000 rpm for 20 min at 4°C. The resultant supernatant was used for the estimation of antioxidative enzymes and was stored at −80°C for analysis. The method of Aebi (1974) was used for the determination of catalase (CAT) activity. Briefly, potassium phosphate buffer (50 mM) and plant extract were used in the reaction (3 mL). To start the reaction, 10 mM H₂O₂ was added. 1 unit of CAT is defined as the number of enzymes, which release 1/2 of peroxide oxygen from 10 mM H₂O₂ solution in 100 s at 25°C. Superoxide dismutase (SOD) activity was measured according to the method of Beyer and Fridovich (1987). The reaction mixture (30 mL) for the determination of SOD was composed of potassium phosphate buffer (50 mM), methionine (9.9 mM), nitroblue tetrazolium (57 mM), and plant extract. The reaction mixture was started by light illumination. One unit of SOD is defined as the enzyme which causes a 50% decrease of SOD inhabitable reduction.

**Determination of Proline Content**

To determine proline content, dry leaf samples (0.5 g) were extracted in sulfosalicylic acid (3%) and then filtered (Bates et al., 1973). Afterward, 2 mL of leaf extract was mixed in ninhydrin solution (2 mL) and glacial acetic acid (2 mL). Plant samples were incubated at 100°C for 1 h and cooled in an ice bath. Four milliliters of toluene was vigorously added to the mixture. The absorbance value was calibrated at 520 nm using a spectrophotometer. Proline content was estimated using a standard curve.

**Determination of Cadmium Content**

Following Cd treatment, *P. vulgaris* seedlings were rinsed with deionized H₂O. Seedlings were dried at 80°C for 24 h. The plant material was digested in HNO₃:HClO₄. About 1 g plant sample was homogenized in 50 mM Tris (hydroxymethyl) aminomethane (Tris–HCl), 250 mM sucrose, and 1 mM DL-dithiothreitol. The homogenous mixture obtained was centrifuged at 20,000 rpm for 30 min. The supernatant was used in the reaction (3 mL). To start the reaction, 10 mM H₂O₂ was added. 1 unit of CAT is defined as the number of enzymes, which release 1/2 of peroxide oxygen from 10 mM H₂O₂ solution in 100 s at 25°C. Superoxide dismutase (SOD) activity was measured according to the method of Aebi (1974) was used for the determination of catalase (CAT) activity. Briefly, potassium phosphate buffer (50 mM) and plant extract were used in the reaction (3 mL). To start the reaction, 10 mM H₂O₂ was added. 1 unit of CAT is defined as the number of enzymes, which release 1/2 of peroxide oxygen from 10 mM H₂O₂ solution in 100 s at 25°C. Superoxide dismutase (SOD) activity was measured according to the method of Beyer and Fridovich (1987). The reaction mixture (30 mL) for the determination of SOD was composed of potassium phosphate buffer (50 mM), methionine (9.9 mM), nitroblue tetrazolium (57 mM), and plant extract. The reaction mixture was started by light illumination. One unit of SOD is defined as the enzyme which causes a 50% decrease of SOD inhabitable reduction.

**Determination of Nutritional Content**

A flame photometer (Jenway PFP-7) was used for the determination of nutritional content (Mo⁶⁺, Ca⁴⁺, K⁺, Mn⁴⁺) from...
the digested plant samples. K⁺ content was quantified with the help of a standard solution.

**Determination of Gaseous Exchange**
Assessments of gaseous exchange attributes, net CO₂ uptake, net photosynthesis (Pn), stomatal conductance (Gs), and transpiration rate (E), were performed at 10:00 a.m. at 27°C during the daytime on completely expanded leaves using a portable gas exchange system (Holá et al., 2010).

**Determination of Polyamine Activity**
The concentration of hydrogen peroxide produced during polyamine oxidation assisted in the estimation of polyamine oxidase (PAO) activity. The foliage plant sample was mixed with potassium phosphate buffer (0.1 mM at pH 6.5) in an pre-chilled mortar and pestle. The resultant supernatant (0.5 mL) was obtained by centrifugation of this mixture at 10,000 g at 4°C for 20 min followed by mixing with 4-aminoantipyrine/N,N-dimethylaniline solutions (0.4 mL), 0.2 mL horseradish POD (250 U/mL), and 5 mL of K₃PO₄ buffer (100 mM at pH 6.5). For the determination of polyamine oxidase, putrescine (30 mL of 20 mM) was homogenized in enzyme extract to initiate reaction according to Zhao et al. (2004).

**Determination of Nitric Oxide Content**
Nitric oxide activity was quantified using the nitric oxide detection kit (Solarbio Life Science, Beijing, China) following the manufacturer's instructions.

**Estimation of Soluble Protein Content**
Leaf samples (1 g) were extracted using KH₂PO₄ (4 mL at pH 6.8) following centrifugation at 3,600 rpm for 30 min. The resultant supernatant was collected and a 20 µL aliquot of the extract was collected and homogenized with the Bradford color reagent (1 mL). The spectrophotometer calibration was carried out at 595 nm as per the procedure described by Bradford (1976).

**Statistical Analysis**
Data reported in the experiment was mean of 5 replicates. Statistical package XL-STAT was carried out for analysis of variance (ANOVA). Subsequently, Tukey’s test was conducted to determine the significant differences among the values.

**RESULTS**

**Effect of Iron Oxide Nanoparticles and Silicon Nanoparticles on Growth Attributes of Phaseolus vulgaris**
Table 1 shows the role of IONPs and SiNPs, alone or in combination, on the growth of *P. vulgaris* seedlings grown in normal and Cd-contaminated soil. Synergistic application of NPs improved shoot fresh weight and root fresh weight by 39 and 40%, respectively, as compared to IONPs-only treated *P. vulgaris* seedlings grown in non-contaminated soil. When *P. vulgaris* seedlings were exposed to 2 mM CdCl₂, combined treatment of IONPs and SiNPs increased shoot fresh weight and root fresh weight by 45 and 19%, respectively, as compared to SiNP-treated *P. vulgaris* seedlings grown in Cd-contaminated media. Likewise, combined and synergistic treatments involving NPs enhanced root dry weight and shoot dry weight in *P. vulgaris* seedlings grown in normal and Cd-contaminated conditions (Table 1).

**Effect of Iron Oxide Nanoparticles and Silicon Nanoparticles on Leaf Relative Water Content and Net Photosynthetic Rate of Phaseolus vulgaris**
When *P. vulgaris* seedlings were grown in non-contaminated media, combined treatment of IONPs and SiNPs enhanced LRWC content by 9 and 4% in comparison with IONPs-only and SiNPs-only treatment, respectively. A similar trend of net photosynthetic rate was observed in IONPs and SiNPs-treated seedlings grown in normal and Cd-contaminated media.

### Table 1: Effect of IONPs and SiNPs on root fresh weight, shoot fresh weight, root dry weight, shoot dry weight, leaf relative water content, and net photosynthetic rate of *Phaseolus vulgaris* grown in different concentrations of Cd.

| Treatments | Root FW (g plant⁻¹) | Shoot FW (g plant⁻¹) | Root DW (g plant⁻¹) | Shoot DW (g plant⁻¹) | LRWC (%) | Net Photosynthetic rate (µmol m⁻² s⁻¹) |
|------------|----------------------|----------------------|---------------------|---------------------|----------|----------------------------------------|
| Cd0 + IONPs | 4.21 ± 0.45ab | 25.12 ± 1.89b | 0.33 ± 0.0021b | 2.76 ± 0.54bc | 82 ± 6.76 | 1.98 ± 0.35cd |
| Cd0 + SiNPs | 3.76 ± 0.37cd | 23.18 ± 1.46bc | 0.28 ± 0.0031bc | 2.34 ± 0.38bc | 86 ± 5.27 | 2.19 ± 0.76c |
| Cd0 + IONPs + SiNPs | 5.89 ± 0.89a | 34.98 ± 2.87a | 0.56 ± 0.0039a | 3.54 ± 0.39a | 90 ± 5.87 | 3.17 ± 0.38a |
| Cd1 + IONPs | 3.78 ± 0.37c | 21.12 ± 1.78c | 0.21 ± 0.0035cd | 2.09 ± 0.54cd | 78 ± 3.98 | 2.19 ± 0.57bc |
| Cd1 + SiNPs | 2.56 ± 0.87de | 18.98 ± 1.67d | 0.28 ± 0.0076bc | 2.56 ± 0.43c | 80 ± 4.18 | 2.67 ± 0.18bc |
| Cd1 + IONPs + SiNPs | 4.18 ± 0.48b | 24.78 ± 1.09bc | 0.39 ± 0.0072ab | 2.98 ± 0.15ab | 90 ± 5.28 | 3.08 ± 0.24ab |
| Cd2 + IONPs | 2.76 ± 0.27d | 18.23 ± 1.04de | 0.17 ± 0.0043d | 1.89 ± 0.76d | 72 ± 3.67 | 1.09 ± 0.12d |
| Cd2 + SiNPs | 2.23 ± 0.45de | 20.89 ± 1.09cd | 0.26 ± 0.0021c | 2.08 ± 0.45cd | 80 ± 4.27 | 1.89 ± 0.38cd |
| Cd2 + IONPs + SiNPs | 3.89 ± 0.48bc | 23.89 ± 1.34bc | 0.32 ± 0.0028bc | 2.65 ± 0.16c | 92 ± 4.87 | 2.76 ± 0.17b |
| Cd3 + IONPs | 1.89 ± 0.56e | 13.76 ± 1.09f | 0.17 ± 0.0021d | 1.09 ± 0.79e | 63 ± 3.87 | 0.98 ± 0.002d |
| Cd3 + SiNPs | 2.67 ± 0.72de | 15.27 ± 1.38e | 0.13 ± 0.0065d | 1.56 ± 0.37de | 76 ± 4.76 | 1.67 ± 0.21cd |
| Cd3 + IONPs + SiNPs | 3.89 ± 0.51bc | 18.28 ± 1.02de | 0.28 ± 0.0078bc | 2.78 ± 0.28bc | 84 ± 5.98 | 2.09 ± 0.54bc |

Here IONPs, SiNPs, Cd0, Cd1, Cd2, Cd3 indicates iron oxide nanoparticles, silicon nanoparticles, 0 mM CdCl₂, 1 mM CdCl₂, 1.5 mM CdCl₂, 2 mM CdCl₂. Different letters indicate significant difference among the treatments (P < 0.05).
photosynthetic rate was observed in *P. vulgaris* seedlings exposed to different concentrations of CdCl₂ (Table 1).

**Effect of Iron Oxide Nanoparticles and Silicon Nanoparticles on Gas Exchange Parameters of Phaseolus vulgaris**

In the case of *P. vulgaris* seedlings grown in non-contaminated soil, synergistic application of IONPs and SiNPs increased intercellular CO₂ concentration and stomatal conductance by 8 and 18%, respectively, in comparison with alone application of IONP and SiNP treatment. When *P. vulgaris* seedlings were treated with 1 mM CdCl₂, 1.5 mM CdCl₂, and 2 mM CdCl₂, intercellular CO₂ concentration decreased by 20, 29, and 43%, respectively, as compared to Cd₀ treatment. Synergistic application of IONP and SiNP increased intercellular CO₂ concentration in *P. vulgaris* seedlings grown in 1.5 mM CdCl₂ by 22 and 7%, respectively, in comparison with alone treatments of IONPs and SiNPs. Likewise, synergistic application of IONP and SiNP enhanced stomatal conductivity in *P. vulgaris* seedlings grown in Cd₀-treatment and Cd-contaminated soil (Figure 1).

**Effect of Iron Oxide Nanoparticles and Silicon Nanoparticles on Malondialdehyde Content and Electrolyte Leakage in Phaseolus vulgaris**

Figure 2 shows that the application of IONPs and SiNPs reduced MDA content and EL leakage in *P. vulgaris* seedlings grown in non-contaminated and Cd-polluted soil. Synergistic application
of IONPs and SiNPs reduced MDA content by 50 and 20% as compared to individual application of IONPs and SiNPs, respectively, in *P. vulgaris* seedlings grown in 1.5 mM CdCl₂-contaminated soil.

Electrolyte leakage was also reduced in *P. vulgaris* seedlings treated with combined application of IONPs and SiNPs, in comparison with individual treatments. In the case of *P. vulgaris* seedlings grown in 2 mM CdCl₂, combined application of IONPs and SiNPs reduced EL by 56 and 21%, respectively, in comparison with individual treatments of IONPs and SiNPs (Figure 2).

**Effect of Iron Oxide Nanoparticles and Silicon Nanoparticles on Polyamine Content in *Phaseolus vulgaris* Seedlings**

Figure 3 shows the effect of IONPs and SiNPs on spermidine (Spd) and putrescine (Put) content of *P. vulgaris* seedlings grown in normal and Cd-contaminated soil. Application of IONPs and SiNPs, alone or in combination, escalated Spd and Put content in *P. vulgaris*. Combined treatment of IONPs and SiNPs enhanced Spd content by 91% as compared to individual application of SiNPs in *P. vulgaris* seedlings grown in 1 mM CdCl₂. Likewise, Put content also increased when *P. vulgaris* seedlings were treated with IONPs and SiNPs in combination. The combined application of IONPs and SiNPs enhanced Put content by more than onefold in comparison with SiNPs-only treatment in *P. vulgaris* seedlings grown in 2 mM CdCl₂.

**Effect of Iron Oxide Nanoparticles and Silicon Nanoparticles on Nitric Oxide and Proline Content of *Phaseolus vulgaris***

Nitric oxide and proline (Pro) content are considered as stress markers in plants. During the current study, IONPs and SiNPs enhanced nitric oxide and proline
content in *P. vulgaris* seedlings grown in normal and Cd-contaminated soil. In the case of *P. vulgaris* seedlings grown in control conditions, synergistic application of IONPs and SiNPs enhanced NO content by 44 and 50%, respectively, in comparison with individual application of IONPs and SiNPs. When *P. vulgaris* seedlings were exposed to 2 mM CdCl$_2$, combined application of IONPs and SiNPs enhanced NO content by more than onefold and 60% as compared to IONPs-only and SiNPs-only treatment. Similarly, IONPs and SiNPs also enhanced proline content in *P. vulgaris* seedlings grown in normal and Cd-contaminated conditions (Figure 4).

**Effect of Iron Oxide Nanoparticles and Silicon Nanoparticles on the Activity of Antioxidant Enzymes in *Phaseolus vulgaris***

Figure 5 explains the role of IONPs and SiNPs on the activity of SOD and CAT in *P. vulgaris* seedlings grown in control and Cd-contaminated conditions. In the case of *P. vulgaris* seedlings grown in Cd0 treatment, combined application of IONPs and SiNPs improved the activity of SOD by 41 and 71%, respectively, in comparison with IONPs-only and SiNPs-only treatment. Synergistic treatment of IONPs and SiNPs enhanced
SOD content by 72 and 90% as compared to individual treatments of IONPs and SiNPs, respectively, in *P. vulgaris* seedlings grown in 2 mM CdCl$_2$. Likewise, combined treatment of IONPs and SiNPs enhanced CAT activity in *P. vulgaris* seedlings grown in normal and CdCl$_2$-contaminated conditions. The combined application of IONPs and SiNPs escalated CAT activity by 71 and 50%, as compared to IONPs-only and SiNPs-only treatment, respectively, in *P. vulgaris* seedling exposed to 2 mM CdCl$_2$.

**Effect of Iron Oxide Nanoparticles and Silicon Nanoparticles on Cadmium Uptake of *Phaseolus vulgaris***

Table 2 shows that NP treatment reduced Cd uptake in *P. vulgaris* seedlings grown in CdCl$_2$-contaminated soil. Synergistic application of IONPs and SiNPs reduced Cd uptake by 68% in *P. vulgaris* seedling grown in 2 mM CdCl$_2$.

**Effect of Iron Oxide Nanoparticles and Silicon Nanoparticles on Photosynthetic Pigmentation of *Phaseolus vulgaris***

Table 3 shows the role of IONPs and SiNPs on photosynthetic pigmentation of *P. vulgaris* grown in normal and Cd toxic conditions. IONPs and SiNPs orchestrated carotenoids content, Chl *a*, Chl *b*, and total Chl content in *P. vulgaris* seedlings.

**Effect of Iron Oxide Nanoparticles and Silicon Nanoparticles on the Nutritional Content of *Phaseolus vulgaris***

Table 4 describes the role of IONPs and SiNPs on the nutritional content of *P. vulgaris* seedlings grown in normal and different concentrations of CdCl$_2$. A combined application of IONPs and SiNPs increased K$^+$ content by 31 and 24%, respectively, as...
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FIGURE 5 | Effect of IONPs and SiNPs on SOD and CAT activity of \textit{P. vulgaris} grown in different concentrations of Cd. Here IONPs, SiNPs, Cd0, Cd1, Cd2, and Cd3 indicate iron oxide nanoparticles, silicon nanoparticles, 0 mM CdCl$_2$, 1 mM CdCl$_2$, 1.5 mM CdCl$_2$, and 2 mM CdCl$_2$. Different letters indicate significant difference among the treatments ($P \leq 0.05$).

compared to IONPs-only and SiNPs-only treated \textit{P. vulgaris} seedlings grown in non-contaminated potted soil.

**DISCUSSION**

Cadmium toxicity reduces the ability of plants to absorb key nutrients (Mg, Ca, P, K) and water content from soil (Yang et al., 2020). Potassium is considered as crucial for plant growth, development, and metabolomics (Hasanuzzaman et al., 2018) because it alleviates abiotic stresses (Raza et al., 2021) by restricting transportation and accumulation of heavy metals in plants (Kerchev et al., 2020). Additionally, potassium not only immobilizes heavy metal content (Liu et al., 2018) but also behaves antagonistically to Cd and detoxifies Cd within plants (Shamsi et al., 2008). Cadmium is a non-essential metal and is involved in the hindrance of plant growth (Shah et al., 2020a) by causing a reduction in water content, biomass, fresh and dry weight of plants, as well as disruption in redox reaction (Raza et al., 2020). Potassium application stabilizes chlorophyll architecture, which leads to the regulation of photosynthate production (Hasanuzzaman et al., 2018). It was also documented that K$^+$ application enhanced growth and yield in apple dwarf rootstock seedlings (Xu et al., 2020) via Cd assimilation by increased accumulation of K$^+$ in plants (Norvell et al., 2000). In addition, the increase in K$^+$ enhanced the activity of antioxidant enzymes and reduced MDA content in plants exposed to Cd toxicity. The results of the present study also indicated that synergistic application of IONPs and SiNPs enhanced K$^+$ content in \textit{P. vulgaris} seedlings grown in normal and Cd-contaminated soil. In the present study, IONPs and SiNPs treatment increased LRWC in \textit{P. vulgaris} seedling
(Table 1). Before, it was also reported that SiNP treatment enhanced gas exchange characteristics in plants exposed to Cd-contaminated media (Ur Rahman et al., 2021). Cadmium stress destabilized photosynthetic apparatus, yet IONPs and SiNPs application reduced injury to photosynthetic machinery. Iron oxide nanoparticles increased root and shoot length in Arachis hypogaea plants (Rizwan et al., 2019). In the present research, it was noted also that that synergistic application of IONPs and SiNPs increased photosynthesis and gas exchange characteristics in P. vulgaris seedlings.

Proline is crucial for the sustenance of metabolomics in plants (Ahmad et al., 2019). It was noted in the present investigation that P. vulgaris seedlings treated with IONPs and SiNPs showed an increased level of proline under Cd stress. The increased level of proline in NP-treated plants might lead to activation of proline synthesizing genes involved in the regulation of water content in plants.

Membrane stability is an essential characteristic required for the effective survival of plants under abiotic stress since damage to it will lead to cellular death. MDA is an indicator of cellular damage and membrane stability (Shah et al., 2020b). Under stress conditions, the generation of MDA and peroxidation of lipids increased causing damage to plants. In this study, Cd stress amplified MDA and EL in P. vulgaris plants grown in Cd-contaminated soil. Cadmium decreases the stability of membranous molecules due to the enhanced accumulation of ROS. Previous researchers revealed the role of IONPs and SiNPs in alleviating abiotic stresses in plants.

**TABLE 2** | Effect of IONPs and SiNPs on root Cd content, shoot Cd content, translocation factor, and metal tolerance index of Phaseolus vulgaris grown in different concentrations of Cd.

| Treatments                        | Root (µg g⁻¹ DW) | Shoot (µg g⁻¹ DW) |
|-----------------------------------|------------------|------------------|
| Cd0 + IONPs                       | ND               | ND               |
| Cd0 + SiNPs                       | ND               | ND               |
| Cd0 + IONPs + SiNPs               | ND               | ND               |
| Cd1 + IONPs                       | 0.013 ± 0.002    | 0.05 ± 0.0002    |
| Cd1 + SiNPs                       | 0.014 ± 0.003    | 0.04 ± 0.0001    |
| Cd1 + IONPs + SiNPs               | 0.005 ± 0.004    | 0.002 ± 0.0003   |
| Cd2 + IONPs                       | 0.017 ± 0.001    | 0.009 ± 0.0001   |
| Cd2 + SiNPs                       | 0.015 ± 0.003    | 0.007 ± 0.0004   |
| Cd2 + IONPs + SiNPs               | 0.019 ± 0.008    | 0.005 ± 0.0002   |
| Cd3 + IONPs                       | 0.021 ± 0.005    | 0.003 ± 0.0005   |
| Cd3 + SiNPs                       | 0.041 ± 0.0003   | 0.006 ± 0.0007   |
| Cd3 + IONPs + SiNPs               | 0.049 ± 0.002    | 0.029 ± 0.0006   |

Here IONPs, SiNPs, Cd0, Cd1, Cd2, Cd3 indicates iron oxide nanoparticles, silicon nanoparticles, 0 mM CdCl₂, 1 mM CdCl₂, 1.5 mM CdCl₂, 2 mM CdCl₂.

**TABLE 3** | Effect of IONPs and SiNPs on carotenoids, total chlorophyll content, Chib, and Chla of Phaseolus vulgaris grown in different concentrations of Cd.

| Treatments                        | Carotenoids        | Total Chlorophyll | Chlb | Chla |
|-----------------------------------|--------------------|-------------------|------|------|
| Cd0 + IONPs                       | 4.87 ± 0.89ab      | 1.42 ± 0.071a     | 0.55 ± 0.032ab | 0.87 ± 0.043 |
| Cd0 + SiNPs                       | 3.78 ± 0.76b       | 1.10 ± 0.032bc    | 0.34 ± 0.026c  | 0.76 ± 0.031 |
| Cd0 + IONPs + SiNPs               | 4.93 ± 0.51a       | 1.19 ± 0.024bc    | 0.65 ± 0.016a  | 0.54 ± 0.017 |
| Cd1 + IONPs                       | 3.23 ± 0.47bc      | 0.81 ± 0.027c     | 0.35 ± 0.034bc | 0.46 ± 0.026 |
| Cd1 + SiNPs                       | 3.02 ± 0.39c       | 0.66 ± 0.046      | 0.28 ± 0.026cd | 0.38 ± 0.027 |
| Cd1 + IONPs + SiNPs               | 3.16 ± 0.23bc      | 1.3 ± 0.037b      | 0.41 ± 0.017b  | 0.89 ± 0.031 |
| Cd2 + IONPs                       | 3.09 ± 0.42bc      | 0.78 ± 0.045c     | 0.31 ± 0.015cd | 0.47 ± 0.025 |
| Cd2 + SiNPs                       | 2.89 ± 0.25cd      | 0.65 ± 0.067cd    | 0.23 ± 0.019cd | 0.42 ± 0.027 |
| Cd2 + IONPs + SiNPs               | 3.87 ± 0.31ab      | 0.96 ± 0.047bc    | 0.37 ± 0.023bc | 0.59 ± 0.015 |
| Cd3 + IONPs                       | 2.78 ± 0.18cd      | 0.79 ± 0.097cd    | 0.24 ± 0.021cd | 0.56 ± 0.036 |
| Cd3 + SiNPs                       | 2.67 ± 0.24cd      | 0.65 ± 0.078cd    | 0.21 ± 0.054d  | 0.44 ± 0.045 |
| Cd3 + IONPs + SiNPs               | 3.54 ± 0.26bc      | 1.13 ± 0.037ab    | 0.38 ± 0.018bc | 0.75 ± 0.029 |

Here IONPs, SiNPs, Cd0, Cd1, Cd2, Cd3 indicates iron oxide nanoparticles, silicon nanoparticles, 0 mM CdCl₂, 1 mM CdCl₂, 1.5 mM CdCl₂, 2 mM CdCl₂. Different letters indicate significant difference among the treatments (P < 0.05).

**TABLE 4** | Effect of IONPs and SiNPs on Mo⁺, Ca⁺, K⁺, and Mn⁺ of Phaseolus vulgaris grown in different concentrations of Cd.

| Treatments                        | Mo⁺ content (meq. g⁻¹ DW) | Ca⁺² content (meq. g⁻¹ DW) | K⁺ content (meq. g⁻¹ DW) | Mn⁺² content (meq. g⁻¹ DW) |
|-----------------------------------|---------------------------|----------------------------|--------------------------|----------------------------|
| Cd0 + IONPs                       | 21.78 ± 1.87ab            | 13.56 ± 1.03b              | 14.87 ± 1.02bc           | 30.89 ± 2.56b              |
| Cd0 + SiNPs                       | 17.56 ± 1.08cd            | 15.78 ± 1.09ab             | 15.76 ± 1.13b            | 25.78 ± 1.47c              |
| Cd0 + IONPs + SiNPs               | 27.98 ± 1.56a             | 19.87 ± 1.27a              | 19.56 ± 1.07a            | 45.98 ± 2.85a              |
| Cd1 + IONPs                       | 17.98 ± 1.08c             | 10.89 ± 1.07cd             | 12.56 ± 1.23cd           | 22.65 ± 1.45cd             |
| Cd1 + SiNPs                       | 15.82 ± 1.23d             | 11.25 ± 1.12bc             | 13.76 ± 1.09c            | 18.65 ± 1.38de             |
| Cd1 + IONPs + SiNPs               | 20.56 ± 1.34b             | 15.89 ± 1.06ab             | 16.78 ± 1.56ab           | 24.98 ± 2.45cd             |
| Cd2 + IONPs                       | 13.98 ± 1.07d             | 10.46 ± 1.18c              | 9.08 ± 0.97d             | 14.76 ± 1.43e              |
| Cd2 + SiNPs                       | 14.89 ± 1.04de            | 11.34 ± 1.14bc             | 11.57 ± 1.09cd           | 16.34 ± 1.28de             |
| Cd2 + IONPs + SiNPs               | 19.78 ± 1.37bc            | 12.45 ± 1.09bc             | 14.27 ± 1.06bc           | 21.59 ± 1.92d              |
| Cd3 + IONPs                       | 10.34 ± 1.08f             | 9.56 ± 1.07de              | 7.65 ± 0.67ef            | 12.98 ± 1.03ef             |
| Cd3 + SiNPs                       | 12.87 ± 1.17e             | 11.67 ± 1.56d              | 10.78 ± 1.08e            | 14.45 ± 1.78e              |
| Cd3 + IONPs + SiNPs               | 13.89 ± 1.67de            | 14.87 ± 1.45cd             | 14.87 ± 1.09de           | 25.89 ± 2.67c              |

Here IONPs, SiNPs, Cd0, Cd1, Cd2, Cd3 indicates iron oxide nanoparticles, silicon nanoparticles, 0 mM CdCl₂, 1 mM CdCl₂, 1.5 mM CdCl₂, 2 mM CdCl₂. Different letters indicate significant difference among the treatments (P < 0.05).
The total antioxidant activity of a plant is a good indicator of how well the plant's antioxidant system is functioning in the presence of oxidative stress. Plants have a variety of antioxidative defense systems to deal with oxidative stress, particularly those produced by the generation of excess ROS as a result of abiotic stress. Superoxide dismutase (SOD) is the first line of defense that is involved in the conversion of superoxide anion to peroxide. Catalase (CAT) is also a crucial antioxidant that plays an important role in plants' defensive approach to abiotic stresses. This enzyme is involved in the conversion of H$_2$O$_2$ to O$_2$ and H$_2$O. Current research reported that IONPs and SiNPs enhanced the activity of CAT in _P. vulgaris_ seedlings exposed to Cd stress.

Nanoparticles reduced MDA levels, while they increased peroxidase (POD) and SOD activity in metal-stressed wheat plants (Sardar et al., 2022). Additionally, osmoregulators control the structure of organelles and macromolecules in plants by adjusting osmosis and aid in the reduction of Cd-induced toxicity in plants (Thind et al., 2021). In the present study, we also noticed that Cd inhibited the production of total soluble proteins and proline concentration in _P. vulgaris_ seedlings. The findings of the present study were in line with the findings of Azizi et al. (2021). Application of NPs in the present study enhanced proline accumulation in Cd treated _P. vulgaris_ seedlings. Enhanced accumulation of proline and soluble sugar may be due to augmented proline playing some vital roles in scavenging ROS, adjusting osmotic equilibrium and determining the membrane attributes in plants exposed to stress (Aswani et al., 2019). Many authors investigated the positive effect of NPs on the enhancement of proline accumulation under stress conditions in broad bean plants in addition to adjusted antioxidant enzyme activities, soluble sugars, and amino acids (Sadak and Bakry, 2020). Furthermore, Azimi et al. (2021) claimed that NP supply can decline salt stress in tomatoes by improving photosynthetic machinery, phenolics, and antioxidant enzyme activities and yield as well.

Cadmium phytotoxicity increased the formation of ROS and produced oxidative burst by interfering with the antioxidant defense system (Kamran et al., 2021), which promotes the MDA content due to the high accumulation of lipid peroxidation of the membrane. During the normal metabolism, the plants have established well-organized antioxidant enzyme defense mechanisms to eliminate the ROS (Fan et al., 2020). This is because SOD acts as the first line of defense in plants against ROS. Superoxide dismutase converts the O$_2^-$ to less toxic H$_2$O$_2$, and it forms the first line of defense in the antioxidant system of plants; to this end, CAT scavenges the H$_2$O$_2$ to water and oxygen molecules (Orabi and Abou-Hussein, 2019). The ameliorative influence of the NPs on Cd stress was also assessed by Hussain et al. (2018) in the wheat plant. This apparently will promote antioxidant impairments by decreasing H$_2$O$_2$ and MDA content and enhancing the activities of SOD, CAT, and increased tolerance against abiotic toxicity (Sharma et al., 2019).

**CONCLUSION**

Cadmium toxicity enhanced oxidative stress in _P. vulgaris_ seedlings. Application of IONPs and SiNPs escalated the activity of antioxidant enzymes besides incrementation in K$^+$ content. Additionally, synergistic application of IONPs and SiNPs increase NO content and Pro content in _P. vulgaris_ seedlings grown in normal and CdCl$_2$-polluted soil. Cd-stress alleviation is credited to increased activity of antioxidant enzymes and maintenance of PAs and K$^+$ metabolism. It is further added that mechanisms and molecules involved in the interaction of different NPs can be exploited for the mitigation of numerous stresses in plants.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

**AUTHOR CONTRIBUTIONS**

LK contributed toward designing the research and conceptualization. AU and AAS performed the experiments. AAS performed the statistical analysis. NAY and ZS contributed to the review and drafting. LR contributed to writing the manuscript. AR and TJ drafted the manuscript. MHS and SA were involved in funding acquisition and writing. All authors contributed to the article and approved the submitted version.

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