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# Modulation of Cd carriers by innovative nanocomposite (Ca+Mg) and Cd-resistance microbes (*Bacillus pumilus*): a mechanistic approach to enhance growth and yield of rice (*Oryza sativa* L.)

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Cadmium (Cd) is a well-known pollutant in agricultural soil, affecting human health through the food chain. To combat this issue, Ca + Mg (25 mg $L^{-1}$) nanocomposite and *Bacillus pumilus*, either alone or combined, were applied to rice plants under Cd (5 mg $kg^{-1}$, 10 mg $kg^{-1}$) contamination. In our study, growth and yield traits demonstrated the beneficial influence of Ca + Mg and *B. pumilus* application in improving rice defense mechanism by reducing Cd stress. Combined Ca + Mg and *B. pumilus* application increased SPAD (15), total chlorophyll (18), chlorophyll a (11), chlorophyll b (22), and carotenoids (21%) with Cd (10 mg $kg^{-1}$), compared to the application alone. Combined Ca + Mg and *B. pumilus* application significantly regulated MDA (15), $H_2O_2$ (13), EL (10), and $O_2^{\bullet-}$ (24%) in shoots under Cd (10 mg $kg^{-1}$), compared to the application alone. Cd (10 mg $kg^{-1}$) increased the POD (22), SOD (21), APX (12), and CAT (13%) in shoots with combined Ca + Mg and *B. pumilus* application, compared to the application alone. Combined Ca + Mg and *B. pumilus* application significantly reduced Cd accumulation in roots (22), shoots (13), and grains (20%) under Cd (10 mg $kg^{-1}$), compared to the application alone. Consequently, the combined application of Ca + Mg and *B. pumilus* is a sustainable solution to enhance crop production under Cd stress.

KEYWORDS

**calcium, magnesium, nanocomposite, rice, *Bacillus pumilus***


GRAPHICAL ABSTRACT

## Highlights

- Cd toxicity inhibited rice growth, yield, and antioxidative activity more than control.
- Cd toxicity decreased the nutrient uptake by increasing Cd accumulation.
- Ca + Mg + *B. pumilus* application enhanced rice growth and regulated the oxidative stress, compared to the application alone.
- Ca + Mg + *B. pumilus* application reduced Cd uptake and increased macro and micronutrients in shoots and grains, compared to the application alone.

## 1 Introduction

Heavy metal contamination, such as cadmium (Cd), is a widespread environmental hazard that has gained significant attention in agricultural soil (Alengebawy et al., 2021; Ali et al., 2022a; Zulfiqar et al., 2023a). Cd is rated 7th on the list of hazardous compounds due to its carcinogenic properties, according to the US Agency for Toxic Substances and Disease Registry (ASTDR, 2021; Zulfiqar et al., 2022a). Due to inadequate crop production strategies, Cd has caused food safety issues in recent years. Plants rapidly uptake, accumulate, and translocate Cd into different parts, particularly edible ones, triggering food safety issues (Liu et al., 2010; Zeb et al., 2022; Zulfiqar et al., 2022b). Rice is a staple crop with an annual production of 754.6 million tons, feeding half of the world's population and representing a vital component of the global agricultural economy (FAO, 2017). Cd toxicity inhibits plant height, root length, leaf area, and the number of leaves per plant in rice (Song et al., 2015). Cd significantly reduces the growth and agronomical attributes of rice (Wang et al., 2014; Li et al., 2022). Numerous studies reported that Cd stress enhances oxidative stress through reactive oxygen species (ROS) production in rice seedlings (Srivastava et al., 2014; Ayyaz et al., 2022; Nazir et al., 2022). Therefore, it is essential to develop an effective remediation strategy to deal with Cd-contaminated soil. Various physical, chemical, and biological remediation techniques have been adopted for the removal of heavy metal contamination. These techniques are often unrealistic due to poor efficiency (Nafees et al., 2018; Hou et al., 2020; Yang et al., 2020). Hence, there is a need to develop cost-effective, eco-friendly, and innovative approaches to limit the impact of heavy metal pollution on global food safety (Chen and Li, 2018; Li et al., 2022).

Nanotechnology has garnered significant attention in the modern era, as this field has radically transformed modern science and is expanding exponentially (Bhardwaj et al., 2022). Recently, nanoparticles (NPs) have significantly enhanced plant growth and development by reducing heavy metal uptake in plants (Nafees et al., 2020; Babu et al., 2022; Ulhassan et al., 2022; Khalid et al., 2023; Nafees et al., 2024a). NPs mitigate stress by regulating phytohormones (Maqsood et al., 2023). According to a study, copper (Cu) NPs enhanced the growth and agronomical attributes of wheat by mitigating Cd toxicity (Noman et al., 2020). The foliar application of nano zinc (Zn) and iron (Fe) has shown a beneficial effects in *Rosmarinus officinalis* (Hassanpouraghdam et al., 2020). Similarly, the foliar application of ceric oxide ($CeO_2$) and copper oxide (CuO) NPs enhanced the fresh weight, yield, and nutritional quality of cucumber (Hong et al., 2016).

Calcium (Ca) and magnesium (Mg) are ubiquitous metal elements in the Earth's crust, and their ionic forms ($Ca^{2+}$ and $Mg^{2+}$) in soil are vital plant nutrients. Mg plays a fundamental role in regulating physical and biochemical processes, and its deficiency is a limiting factor for crop production (Ishfaq et al., 2021, 2022). $Ca^{2+}$ and $Mg^{2+}$ are absorbed by roots and transported to shoots via xylem. CaO NPs significantly enhanced plant biomass, and enzymatic and non-enzymatic antioxidative activity due to a substantial decrease in ROS species under Cd toxicity (Nazir et al., 2022). Li et al. (2022) reported that $Ca^{2+}$ and $Mg^{2+}$ treatment boosted rice growth and yield by reducing uptake and accumulation of Cd in grains, roots, stems, and leaves. Limited research has focused on investigating the influence of Ca and Mg

NPs on plant–soil systems, particularly regarding soil microbial interactions with plants.

Like NPs, microbes play a vital role in plant growth by reducing the uptake and accumulation of heavy metals (Hassan et al., 2017; Ali et al., 2022b; Zulfiqar et al., 2023b). *Bacillus pumilus* is a growth-promoting bacterium in plants. *B. pumilus* reduces the uptake of cadmium and promotes plant height and photosynthetic pigments in rapeseed (*Brassica napus* L.) (Masood et al., 2020). Similarly, *Bacillus* spp. enhances the activity of ROS-scavenging enzymes such as POD, SOD, CAT, and APX, and boosts maize tolerance to Zn and Cu stress (Shahzad et al., 2021). Currently, no studies have been conducted on the combined effect of Ca + Mg nanocomposite and microorganisms on rice growth. Furthermore, the risk assessment of their toxicity to rice and soil is still in its early stages. Meanwhile, the combination of nanoparticles and microbial strain inoculation has recently attracted significant attention in agriculture due to their greater efficacy in alleviating heavy metal stress (Babu et al., 2022; Ahmed et al., 2023).

Therefore, a novel Ca + Mg nanocomposite was synthesized and the specific objectives of the current study were: a) to evaluate the individual and combined effect of Ca + Mg nanocomposite and *B. pumilus* on rice yield and growth; b) investigate the alleviating combined role of the Ca + Mg nanocomposite and microbes on oxidant and antioxidant enzymatic activity; c) to analyze the individual and combined effect of the Ca + Mg nanocomposite and *B. pumilus* on Cd uptake and accumulation in rice. Thus, the current study could offer an economically feasible alternative fertilizer that promotes sustainable agricultural crop production with higher nutritional value.

# 2 Material and methods

## 2.1 Soil collection and scrutiny

The soil was collected from the fields of the University of Agriculture, Faisalabad. Soil was air-dried and sieved through a 2 mm sieve. The soil used in this study was sandy clay loam according to Bouyoucos (1962). The electrical conductivity (EC) was 1.85 dS $m^{-1}$, and the pH (7.68) of the soil extract was measured using appropriate methods. Available Cd (0.07 mg $kg^{-1}$) was measured following the standard procedure of Amacher (1996). Soil organic carbon was assessed by following the Walkley–Black protocol, while calcium carbonate, total nitrogen, available phosphorous, extractable potassium, and cation exchange capacity were 3.16 g $kg^{-1}$, 3.2%, 0.089%, 6.1 mg $kg^{-1}$, 92 mg $kg^{-1}$, 10.4 cmol $_{(+)}$ $kg^{-1}$, respectively, using appropriate methods. Similarly, Zn, Mn, and Fe were 5.13 mg $kg^{-1}$, 4.97 mg $kg^{-1}$, and 53.74 mg $kg^{-1}$, respectively, determined using the calcimeter method (Moodie et al., 1959; Jackson, 1962).

## 2.2 Ca + Mg nanocomposite synthesis

The calcium and magnesium nanocomposite was synthesized following the method of Das et al. (2018) using calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$) and magnesium nitrate tetrahydrate ($Mg(NO_3)_2 \cdot 4H_2O$). Separate solutions of calcium nitrate tetrahydrate (0.1 M) and magnesium nitrate tetrahydrate (0.1 M) were prepared. Both solutions were mixed, and sodium hydroxide (NaOH) pellets were added to maintain a pH 11.5. The mixture was stirred on a hotplate at 90°C, 120 g for 6 h. The formation of the Ca and Mg nanocomposite was confirmed by visually observing the color change of the solution. After 6 h, the solution was cooled to room temperature and centrifuged at 7,000*g*, 25°C for 5 min. The obtained residue was then washed several times with double-distilled water, dried in an oven at 80°C for 24 h, and ground using a mortar and pestle. The nanocomposite was calcined at 500°C for 3 h using a furnace tube to homogenize and remove impurities. X-ray diffraction (XRD) patterns and Fourier transform infrared (FT-IR) spectra were analyzed using PANalytical B·V. (Netherlands) and PerkinElmer, respectively. The surface morphology of the nanocomposite was examined by scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDX) and transmission electron microscopy (TEM), as shown in Figure 1.

## 2.3 Seeds inoculation with *B. pumilus*

The isolated strain *B. pumilus* (KF859972) was collected from the Department of Microbiology, GC University, Faisalabad, and prepared following Zeng et al. (2020). Nutrient broth (250 mL) simmered on a rotary shaker (150 g, 37°C, 24 h), then collected after centrifugation (10,000*g*, 10 min). The supernatant was discarded, and the residue was washed with sterilized distilled water. Surface-sterilized seeds (hydrogen peroxide 10% $H_2O_2$) were inoculated with bacteria using carboxymethyl cellulose (2%) on a rotary shaker at room temperature (90 g). A mixture of clay and peat moss (1:1 w/w) was used to coat the inoculated seeds. Both normal and inoculated seeds were planted in control and respective treatment conditions according to the treatment plan.

## 2.4 Experimental conditions

A pot experiment was carried out under natural environmental conditions (day–night temperature, 39/32°C, and humidity, 78 ± 4%) in 2022 at the Botanical Garden of Government College University Faisalabad, Pakistan. Treatments includes: Control; Cd 5 mg $kg^{-1}$, Cd 10 mg $kg^{-1}$, Microbes, Cd 5 mg $kg^{-1}$ + Microbes, Cd 10 mg $kg^{-1}$ + Microbes, (Ca + Mg) foliar, Cd 5 mg $kg^{-1}$ + (Ca + Mg) foliar, Cd 10 mg $kg^{-1}$ + (Ca + Mg) foliar, Microbes + (Ca + Mg) foliar, Cd 5 mg $kg^{-1}$ + (Ca + Mg) foliar + Microbes, Cd 10 mg $kg^{-1}$ + (Ca + Mg) foliar + Microbes. Pots were filled with sifted soil (5 kg $pot^{-1}$) spiked with Cd (5 mg $kg^{-1}$ and 10 mg $kg^{-1}$) using cadmium chloride according to the treatment plan. A complete randomized design was used to conduct the experiment in triplicate. Rice seeds were soaked in water for 48 h, inoculated with *B. pumilus* before sowing, and grown in sieved and washed sand. After 20 days of

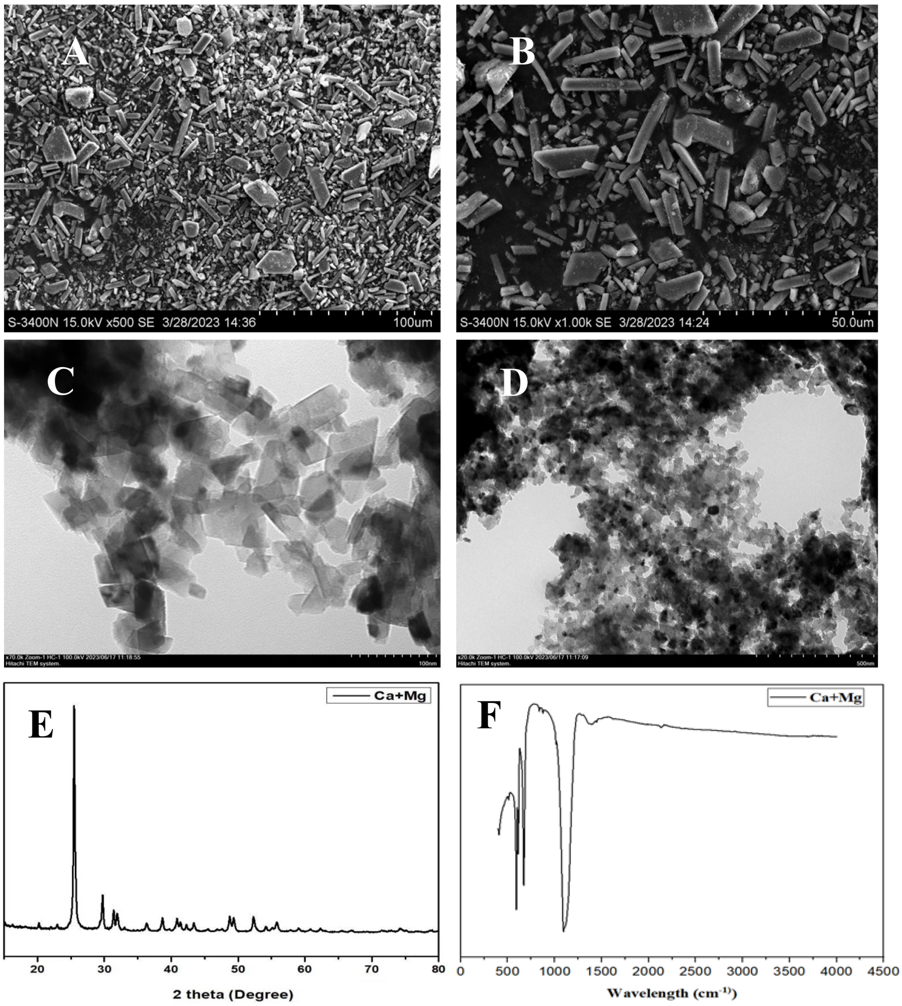


FIGURE 1
SEM of calcium and magnesium (Ca+Mg) **(A, B)**, TEM **(C, D)**, XRD **(E)** and FTIR **(F)**.

germination, the rice plants were transplanted into pots, each containing four healthy plants. The recommended dose of NPK fertilizer was applied to prevent nutrient deficiency. The foliar Ca + Mg nanocomposite was applied after germination. A total of seven foliar applications were sprayed at one-and-a-half week intervals.

## 2.5 Measurement of photosynthetic pigments and gas exchange parameters

SPAD value was measured using an *in situ* SPAD meter. Photosynthetic pigments such as chlorophyll a and b, total chlorophyll, and carotenoids were determined spectrophotometrically in fresh rice leaves (Lichtenthaler, 1987). Acetone 85% (v/v ratio) was used to extract the samples to assess chlorophyll and carotenoid contents. Readings were taken using a spectrophotometer after extraction and centrifugation of samples. Gas exchange parameters (photosynthesis rate, transpiration rate, stomatal conductance, and water use efficiency) were measured during sunlight (12:00 a.m.) in leaves of rice plants using an infrared gas analyzer (IRGA).

## 2.6 Harvesting of plants

Plants were harvested and carefully separated into shoots, roots, leaves, and grains after 120 days of sowing. Growth parameters such as root and shoot lengths (cm), root fresh and dry weights (g), shoot fresh and dry weights (g), spike length, and number of grains were determined. Roots were washed with HCl (0.1%) and distilled water to remove metals. Root, shoot, and grain samples were oven-dried (72 h at 80°C) and crushed into small pieces for further analysis.

## 2.7 Measurement of oxidative stress and antioxidant enzymatic activity

Malondialdehyde (MDA) levels were assessed using the TBA (0.1%) method (Zhang and Kirkham, 1994; Abbas et al., 2017). Electrolyte leakage determination was achieved in two steps. Initial EC was noted after incubating samples for 2 h at 32°C. Second EC was measured for 20 min at 121°C following Dionisio-Sese and Tobita (1998). $H_2O_2$ activity was analyzed as per Jana and Choudhuri (1982).

Samples were crushed in phosphate buffer (PB 5 Mm and pH 6.5) and centrifuged for 20 min. Sulfuric acid (20%) was added after centrifugation and then centrifuged for 15 min. Absorbance was measured at a wavelength of 410 nm using a spectrophotometer. Superoxide radical ($O_2^{\bullet-=}$) contents were measured by obtaining fresh leaf extract in hydroxylamine hydrochloride, titrated with naphthylamine (7 mM) and sulfanilamide (17 mM) (Yang et al., 2011).

Phosphate buffer (PB 0.5, at pH 7.8) was used to homogenize the leaf and root samples for the determination of peroxidase (POD) and superoxide dismutase (SOD) contents (Zhang, 1992). Ascorbate peroxidase (APX) activity was estimated using the Nakano and Asada (1981) protocol, while CAT contents were assessed using the Aebi (1984) method.

## 2.8 Measurement of metabolites

Total soluble proteins (TSP) were measured by homogenizing 0.5 g of fresh leaves in potassium phosphate buffer (50 mM, pH 7.5) following Bradford (1976). Fresh leaves (0.5 g) were crushed in a potassium phosphate buffer solution (50 mM, pH 7.5). Pyridine and acid ninhydrin were used to titrate the supernatant to measure total free amino acids (TFAA) (Hamilton et al., 1943). Total soluble sugars (TSS) were analyzed by homogenizing 0.5 g of fresh leaves in an ethanol and ethanol mixture, and the extract was reacted with an anthrone reagent (Yemm and Willis, 1954). Phenolics were determined by triturating 0.5 g of fresh leaves in acetone, centrifuging at 10,000*g* for 10 min, and then reacting the supernatant with Folin and Ciocalteau's phenol reagent for determination (Wolfe et al., 2003).

## 2.9 Assessment of metal contents and macro and micronutrients

The protocol of Lwalaba et al. (2020) was adopted with slight modification. Briefly, samples were digested in a diacid mixture of $HNO_3$:$HClO_4$ (4:1 v/v) at 140°C on a hotplate. Concentrations of elements such as Ca, Mg, Mn, Zn, Fe, K, and Cd were determined using ICP-MS (iCAP RQ, Thermo Scientific).

## 2.10 Statistical analysis

Data analysis was performed using SPSS version 16.0 (SPSS, Chicago, IL). One-way analysis of variance (ANOVA) was conducted, following the Tukey HSD test to assess significant differences among means.

# 3 Results

## 3.1 Effect of Ca + Mg nanocomposite and *B. pumilus* on growth parameters

Foliar application of Ca + Mg nanocomposite and microbial inoculation showed positive effects on rice growth, physiology, and antioxidant contents under Cd (5 mg $kg^{-1}$, 10 mg $kg^{-1}$) toxicity. The results showed that Cd (5 mg $kg^{-1}$) reduced the length of roots and shoots by 20% and 30%, weight of fresh roots and shoots by 22% and 13%, weight of dry roots and shoots by 19% and 16%, number of tillers and grains by 38% and 19%, and spike length by 21% compared to the control treatment. Similarly, the roots and shoots length were reduced by 44% and 46%, weight of fresh roots and shoots by 42% and 32%, weight of dry roots and shoots by 36% and 37%, number of tillers and grains by 59% and 35%, and spike length by 41% significantly ($p \leq 0.05$) inhibited with Cd (10 mg $kg^{-1}$) compared with control treatment. Meanwhile, the *B. pumilus* significantly ($p \leq 0.05$) increased the length of roots and shoots length by 23% and 22%, weight of fresh roots and shoots by 26% and 10%, wet of dry roots and shoots by 21% and 12%, number of tillers and grains by 20% and 19%, and spike length by 13% compared to the control. Moreover, *B. pumilus* inoculation enhanced the length of the roots and shoots by 3% and 12%, weight of fresh roots and shoots by 10% and 11%, weight of dry roots and shoots by 7% and 11%, number of tillers and grains by 46% and 16%, and spike length by 15% in Cd (5 mg $kg^{-1}$) contamination compared without *B. pumilus* treatment. Under Cd (10 mg $kg^{-1}$) contamination, *B. pumilus* improved the length of roots and shoots by 11% and 19%, weight of fresh roots and shoots by 15% and 19%, weight of dry roots and shoots by 9% and 25%, number of tillers and grains by 75% and 22%, and spike length by 24% compared without *B. pumilus* treatment.

Foliar application of Ca + Mg nanocomposite promoted the length of roots and shoots by 22 and 14%, weight of fresh roots and shoots by 23 and 10%, weight of dry roots and shoots by 18 and 15%, number of tillers and grains by 18 and 20%, and spike length by 26% compared to control treatment. Application of Ca + Mg nanocomposite boosted the length of roots and shoots by 17 and 15%, weight of fresh roots by 9%, weight of dry roots and shoots by 8 and 2%, number of grains by 4%, spike length by 4%, and decreased the weight of fresh shoots by 1% and number of tillers by 3% under Cd (5 mg $kg^{-1}$) contamination compared to Cd (5 mg $kg^{-1}$) + *B. pumilus* treatment. Similarly, Ca + Mg nanocomposite foliar application enhanced the length of roots and shoots by 21% and 18%, weight of fresh roots by 13%, dry roots and shoots weight 17% and 3%, number of grains by 3%, spike length by 4%, while decreasing the weight of fresh shoots by 2%, and no. of tillers by 7% under Cd (10 mg $kg^{-1}$) contamination compared with Cd (10 mg $kg^{-1}$) + *B. pumilus* treatment.

The highest increase was observed with the combined application of Ca + Mg foliar spray and *B. pumilus*. Significantly ($p \leq 0.05$), the combined application of Ca + Mg and *B. pumilus* inoculation boosted length of roots and shoots by 14% and 8%, weight of fresh roots and shoots by 11% and 9%, weight of dry roots and shoots by 8% and 10%, number of tillers and grains by 21% and 16%, and spike length by 21% compared with *B. pumilus* treatment. While, the combined application of Ca + Mg and *B. pumilus* inoculation augmented the length of roots and shoots by 31% and 28%, weight of fresh roots and shoots by 17% and 9%, weight of dry roots and shoots by 15% and 14%, number of tillers and grains by 17% and 19%, and spike length by 23% with Cd (5 mg $kg^{-1}$) contamination as compared to Cd (5 mg $kg^{-1}$) + *B. pumilus* treatment. Similarly, the combined application of Ca + Mg and *B. pumilus* significantly ($p \leq 0.05$) boosted the length of roots and shoots by 43% and 32%, weight of fresh roots and shoots by 27% and 16%, weight of dry roots and shoots by 24 and 14%, number of tillers and grains by 25% and 22%, and spike length by 29% under Cd (10 mg $kg^{-1}$) as compared with Cd (10 mg $kg^{-1}$) + *B. pumilus* treatment.

## 3.2 Effect of Ca + Mg nanocomposite and *B. pumilus* on photosynthetic pigments

The statistical analysis showed that the SPAD values, chlorophyll a, b, total chlorophyll, and carotenoid with Cd (5 mg $kg^{-1}$, 10 mg $kg^{-1}$) toxicity decreased at a significant ($p \leq 0.05$). Results showed that the Cd (5 mg $kg^{-1}$) abridged the SPAD values, chlorophyll a, b, total chlorophyll, and carotenoid contents 12%, 21%, 32%, 25%, and 33%, compared with the control treatment. Similarly, Cd (10 mg $kg^{-1}$) at a significant ($p \leq 0.05$) minimized the SPAD, chlorophyll a, b, total chlorophyll, and carotenoid contents 34%, 36%, 49%, 42%, and 55%, as compared to the control treatment. Besides, compared with the control treatment, *B. pumilus* application boosted SPAD values, chlorophyll a, b, total chlorophyll, and carotenoids 23%, 17%, 13%, 15%, and 17%. Similarly, *B. pumilus* enhanced the SPAD, chlorophyll a, b, total chlorophyll, and carotenoid contents 6%, 12%, 25%, 26%, and 25% with Cd (5 mg $kg^{-1}$) contamination as compared with respective treatment. Under Cd (10 mg $kg^{-1}$) stress, *B. pumilus* inoculation at a significant ($p \leq 0.05$) enhanced the SPAD, chlorophyll a, b, total chlorophyll, and carotenoid contents 17%, 20%, 33%, 26%, and 50% compared with respective treatment.

Ca + Mg nanocomposite increased the SPAD, chlorophyll a, b, total chlorophyll, and carotenoid contents by 10%, 9%, 8%, 10%, and 12% as compared with control. Under Cd (5 mg $kg^{-1}$) contamination, Ca + Mg nanocomposite enhanced the SPAD, chlorophyll a, b, total chlorophyll, and carotenoid contents 5%, 9%, 2%, 5%, and 10% as compared with Cd (5 mg $kg^{-1}$) + *B. pumilus* treatment. Similarly, Ca + Mg nanocomposite application increased the SPAD values, chlorophyll a, b, total chlorophyll, and carotenoid contents by 9%, 6%, 8%, 6%, and 12% with Cd (10 mg $kg^{-1}$) contamination as compared with Cd (10 mg $kg^{-1}$) + *B. pumilus* treatment. Besides this, combined application of microbial inoculation and Ca + Mg nanocomposite significantly enhanced the SPAD, chlorophyll a, b, total chlorophyll, and carotenoid contents. The results showed that the combined Ca + Mg and *B. pumilus* application considerably increased the SPAD, chlorophyll a, b, total chlorophyll, and carotenoid contents 9%, 10%, 15%, 18%, and 11% as compared with *B. pumilus* treatment. However, the combined Ca + Mg and *B. pumilus* application increased the SPAD, chlorophyll a, b, total chlorophyll, and carotenoid contents by 13%, 16%, 15%, 17%, and 20% with Cd (5 mg $kg^{-1}$) contamination as compared with Cd (5 mg $kg^{-1}$) + *B. pumilus* treatment. Similarly, Cd (10 mg $kg^{-1}$) contamination with combined Ca + Mg and *B. pumilus* application at a significant ($p \leq 0.05$) increased the SPAD, chlorophyll a, b, total chlorophyll, and carotenoid contents 15%, 11%, 22%, 18%, and 21% as compared with Cd (10 mg $kg^{-1}$) + *B. pumilus* treatment.

Likewise, the SPAD, chlorophyll, and carotenoid contents, the photosynthesis rate (Pn), transpiration rate (Tr), water use efficiency (WUE), and stomatal conductance were also enhanced with foliar application of Ca + Mg nanocomposite and microbial inoculation under Cd (5 mg $kg^{-1}$, 10 mg $kg^{-1}$) stress. Results showed that the Pn, Tr, WUE, and stomatal conductance were inhibited 20%, 23%, 22%, and 24% with Cd (5 mg $kg^{-1}$) contamination, as compared with control. Similarly, under Cd (10 mg $kg^{-1}$), the Pn, Tr, WUE, and stomatal conductance were reduced 44%, 39%, 42%, and 40%, respectively, compared with the control. While *B. pumilus* inoculation enhanced the Pn, Tr, WUE, and stomatal conductance 12%, 24%, 19%, and 26%, compared with the control. Moreover, *B. pumilus* treatment enhanced the Pn, Tr, WUE, and stomatal conductance 16%, 17%, 21%, and 20% with Cd (5 mg $kg^{-1}$) contamination, compared to without *B. pumilus*. Under Cd (10 mg $kg^{-1}$) contamination, *B. pumilus* inoculation at a significant ($p \leq 0.05$) amplified the Pn, Tr, WUE, and stomatal conductance by 26%, 21%, 33%, and 27%, compared to without *B. pumilus* inoculation. Ca + Mg nanocomposite promoted the Pn, Tr, WUE, and stomatal conductance by 11%, 13%, 10%, and 18% as compared with control. Ca + Mg nanocomposite increased Pn, Tr, WUE, and stomatal conductance by 2%, 5%, 3%, and 9% with Cd (5 mg $kg^{-1}$) contamination compared with Cd (5 mg $kg^{-1}$) + *B. pumilus* treatment. Similarly, foliar application of Ca + Mg nanocomposite enhanced the Pn, Tr, WUE, and stomatal conductance 6%, 3%, 5%, and 1% with Cd (10 mg $kg^{-1}$) contamination, compared with Cd (10 mg $kg^{-1}$) + *B. pumilus* treatment.

The highest increase was observed with the combined application of Ca + Mg and *B. pumilus* inoculation. Further, the combined application of Ca + Mg and *B. pumilus* inoculation enhanced the Pn, Tr, WUE, and stomatal conductance by 17%, 9%, 11%, and 12% at a significant ($p \leq 0.05$) compared to *B. pumilus* treatment. Meanwhile, combined Ca + Mg and *B. pumilus* application increased the Pn, Tr, WUE, and stomatal conductance by 16%, 11%, 13%, and 19% with Cd (5 mg $kg^{-1}$) contamination compared with Cd (5 mg $kg^{-1}$) + *B. pumilus* treatment. Correspondingly, the combined Ca + Mg and *B. pumilus* application significantly ($p \leq 0.05$) promoted the Pn, Tr, WUE, and stomatal conductance by 28%, 10%, 19%, and 16% with Cd (10 mg $kg^{-1}$) contamination compared with Cd (10 mg $kg^{-1}$) + *B. pumilus* treatment.

## 3.3 Effect of Ca + Mg nanocomposite and *B. pumilus* on oxidative stress markers and antioxidant enzymatic activities

Foliar application of Ca + Mg nanocomposite and microbial inoculation showed a significant ($p \leq 0.05$) effect on oxidants and antioxidants enzymatic activity in roots and leaves such as MDA, $H_2O_2$, EL, $O_2^{\bullet-}$, POD, SOD, APX, and CAT with Cd (5 mg $kg^{-1}$, 10 mg $kg^{-1}$) contamination (Figures 2A, B). Results showed that Cd (5 mg $kg^{-1}$) boosted MDA, $H_2O_2$, EL, and $O_2^{\bullet-}$ in roots by 36%, 30%,

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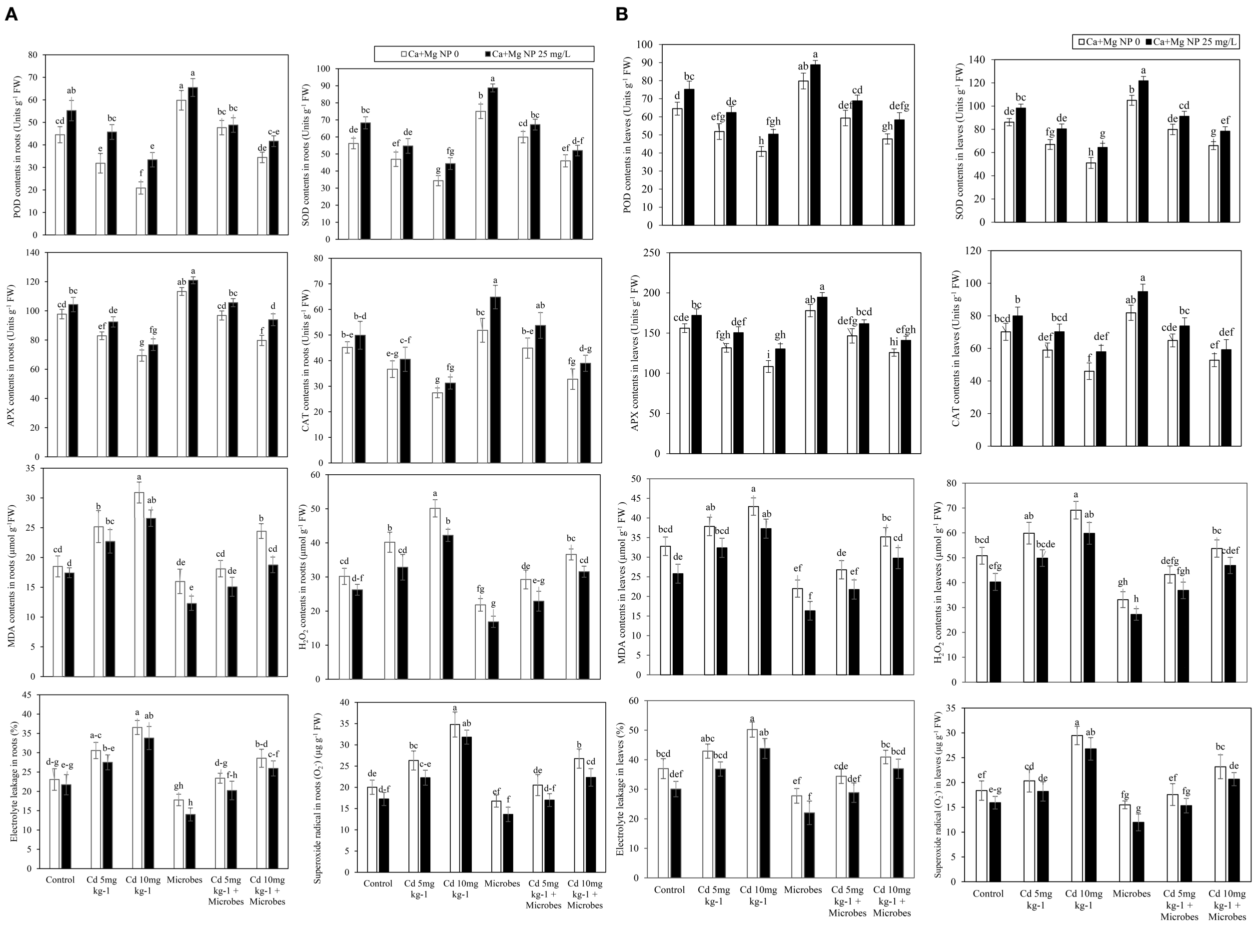


FIGURE 2
**(A)** Alone and combined effect of Ca+Mg nanocomposite (25 mg $L^{-1}$) and inoculation of *Bacillus pumilus* on enzymatic antioxidants POD, SOD, APX and CAT and oxidants EL, MDA, $H_2O_2$ in roots and small letter showed the difference in significance at $p \leq 0.05$ level with mean of three replications. **(B)** Alone and combined effect of Ca+Mg nanocomposite (25 mg $L^{-1}$) and inoculation of *Bacillus pumilus* on enzymatic antioxidants POD, SOD, APX and CAT and oxidants EL, MDA, $H_2O_2$ in shoots and small letter showed the difference in significance at $p \leq 0.05$ level with mean of three replications.

33%, and 31% and leaves by 15%, 18%, 16%, and 32% compared with control. Meanwhile, Cd (5 mg $kg^{-1}$) declined the POD, SOD, APX, and CAT in roots by 28%, 16%, 15%, and 19% and leaves by 20%, 22%, 16%, and 17% compared with control. Similarly, Cd (10 mg $kg^{-1}$) further enhanced the MDA, $H_2O_2$, EL, and $O_2^{\bullet-}$ in roots by 67%, 63%, 58%, and 85% and leaves by 31%, 36%, 35%, and 91%, compared with control. The POD, SOD, APX, and CAT activity in roots by 53%, 39%, 29%, and 39% and leaves by 37%, 41%, 31%, and 34% decreased significantly ($p \leq 0.05$) with Cd (10 mg $kg^{-1}$) compared with control. Meanwhile, *B. pumilus* inoculation abridged the MDA, $H_2O_2$, EL, and $O_2^{\bullet-}$ contents in roots by 14%, 29%, 23%, and 16% and leaves by 33%, 35%, 25%, and 16% compared with control.

*B. pumilus* inoculation at a significant ($p \leq 0.05$) boosted POD, SOD, APX, and CAT activity in roots by 34%, 35%, 16%, and 15% and leaves by 24%, 22%, 14%, and 17% compared with control. Moreover, *B. pumilus* inoculation diminished the MDA, $H_2O_2$, EL, and $O_2^{\bullet-}$ contents in roots by 28%, 27%, 22%, and 22% and leaves by 29%, 28%, 20%, and 25% with Cd (5 mg $kg^{-1}$) contamination compared without *B. pumilus* inoculation. *B. pumilus* inoculation enhanced the POD, SOD, APX, and CAT in roots by 50%, 28%, 17%, and 22% and leaves by 14%, 19%, 11%, and 10% with Cd (5 mg $kg^{-1}$) contamination, compared to without *B. pumilus* treatment. Under Cd (10 mg $kg^{-1}$) contamination, the *B. pumilus* inoculation significantly ($p \leq 0.05$) inhibited MDA, $H_2O_2$, EL, and $O_2^{\bullet-}$ production in roots by 21%, 27%, 22%, and 21% and leaves by 18%, 22%, 19%, and 24% compared without *B. pumilus* treatment. Similarly, *B. pumilus* inoculation significantly ($p \leq 0.05$) increased POD, SOD, APX, and CAT activity in roots by 65%, 34%, 15%, and 19% and leaves by 17%, 29%, 16%, and 15% under Cd (10 mg $kg^{-1}$) contamination compared without *B. pumilus* treatment.

Ca + Mg nanocomposite further reduced the production of MDA, $H_2O_2$, EL, and $O_2^{\bullet-}$ in roots by 5%, 15%, 6%, and 14% and leaves by 21%, 20%, 18%, and 13% as compared with control. Ca + Mg nanocomposite boosted the POD, SOD, APX, and CAT in roots by 24%, 21%, 7%, and 11% and leaves by 17%, 14%, 10%, and 14% as compared with control. Ca + Mg nanocomposite diminished the MDA, $H_2O_2$, EL, and $O_2^{\bullet-}$ production in roots by 25%, 12%, 17%, and 8% and leaves by 21%, 15%, 7%, and 15% with Cd (5 mg $kg^{-1}$) contamination compared with Cd (5 mg $kg^{-1}$) + *B. pumilus* treatment. Similarly, foliar application of Ca + Mg nanocomposite increased the POD, SOD, APX, and CAT in roots by 4%, 9%, 5%, and 10% and leaves by 5%, 1%, 3%, and 9% with Cd (5 mg $kg^{-1}$) contamination compared with Cd (5 mg $kg^{-1}$) + *B. pumilus* treatment. Similarly, Ca + Mg nanocomposite lessened the production of MDA, $H_2O_2$, EL, and $O_2^{\bullet-}$ in roots by 9%, 15%, 18%, and 9% and leaves by 7%, 11%, 10%, and 13% with Cd (10 mg $kg^{-1}$) contamination compared with Cd (10 mg $kg^{-1}$) + *B. pumilus* treatment. Additionally, Ca + Mg nanocomposite foliar application promoted the POD, SOD, APX, and CAT in roots by 3%, 4%, 4%, and 5% and leaves by 6%, 2%, 4%, and 10% with Cd (10 mg $kg^{-1}$) contamination compared with Cd (10 mg $kg^{-1}$) + *B. pumilus* treatment.

The highest increase was observed with combined Ca + Mg and *B. pumilus* application. Further, the combined application of Ca + Mg foliar spray and *B. pumilus* inoculation significantly ($p \leq 0.05$) inhibited MDA, $H_2O_2$, EL, and $O_2^{\bullet-}$ production in roots by 23%, 22%, 21%, and 19% and leaves by 26%, 18%, 21%, and 23% compared with *B. pumilus* treatment. The combined Ca + Mg foliar spray and *B. pumilus* inoculation augmented the POD, SOD, APX, and CAT activity in roots by 9%, 18%, 7%, and 25% and leaves by 11%, 16%, 9%, and 16% as compared with *B. pumilus* treatment. Combined Ca + Mg foliar spray and *B. pumilus* inoculation inhibited the MDA, $H_2O_2$, EL, and $O_2^{\bullet-}$ in roots by 17%, 22%, 14%, 17%, and leaves by 19%, 15%, 17%, and 16% with Cd (5 mg $kg^{-1}$) contamination as compared with Cd (5 mg $kg^{-1}$) + *B. pumilus* treatment. The combined Ca + Mg foliar spray and *B. pumilus* inoculation increased the POD, SOD, APX, and CAT in roots by 2%, 12%, 9%, and 20% and leaves by 16%, 13%, 10%, and 14% with Cd (5 mg $kg^{-1}$) contamination compared with Cd (5 mg $kg^{-1}$) + *B. pumilus* treatment. Similarly, the combined Ca + Mg foliar spray and *B. pumilus* inoculation further decreased the MDA, $H_2O_2$, EL, and $O_2^{\bullet-}$ in roots by 23%, 14%, 9%, and 23% and leaves by 15%, 13%, 10%, and 24% with Cd (10 mg $kg^{-1}$) as compared with Cd (10 mg $kg^{-1}$) + *B. pumilus* treatment. The combined Ca + Mg foliar spray and *B. pumilus* inoculation enhanced the POD, SOD, APX, and CAT in roots by 21%, 13%, 18%, and 19% and leaves by 22%, 21%, 12%, and 13% with Cd (10 mg $kg^{-1}$) compared with Cd (10 mg $kg^{-1}$) + *B. pumilus* treatment.

## 3.4 Effect of nanocomposite Ca + Mg and *B. pumilus* on metabolites

Foliar application of Ca + Mg nanocomposite and microbial inoculation showed a slight ($p \leq 0.05$) effect on metabolites in roots and leaves such as TSP, TFAA, TSS, and phenolics under Cd (5 mg $kg^{-1}$, 10 mg $kg^{-1}$) toxicity (Figures 3A, B). Results demonstrated that Cd (5 mg $kg^{-1}$, 10 mg $kg^{-1}$) considerably ($p \leq 0.05$) boosted TSP, TFAA, TSS, and phenolics in roots and leaves compared with the control treatment. Application of Ca + Mg nanocomposite and microbial inoculation slightly decreased the metabolites in roots and leaves with Cd (5 mg $kg^{-1}$, 10 mg $kg^{-1}$) contamination. Meanwhile, *B. pumilus* inoculation slightly ($p \leq 0.05$) reduced the TSP, TFAA, TSS, and phenolics contents in roots by 33%, 27%, 12%, and 11% and leaves by 27%, 29%, 14%, and 15% as compared with control. Moreover, *B. pumilus* inoculation decreased the TSP, TFAA, TSS, and phenolics contents in roots by 11%, 19%, 13%, and 10% and leaves by 23%, 21%, 18%, and 13% with Cd (5 mg $kg^{-1}$) contamination compared without *B. pumilus* treatment. Under Cd (10 mg $kg^{-1}$) stress, *B. pumilus* inoculation slightly ($p \leq 0.05$) inhibited the production of TSP, TFAA, TSS, and phenolics in roots by 9%, 27%, 13%, and 6% and leaves by 13%, 31%, 8%, and 5% compared without *B. pumilus* treatment. Ca + Mg nanocomposite reduced the TSP, TFAA, TSS, and phenolics in roots by 22%, 15%, 11%, and 8% and leaves by 20%, 21%, 12%, and 14%, compared to the control treatment. Ca + Mg nanocomposite increased the TSP, TFAA, TSS, and phenolics in roots by 3%, 9%, 4%, and 3% and

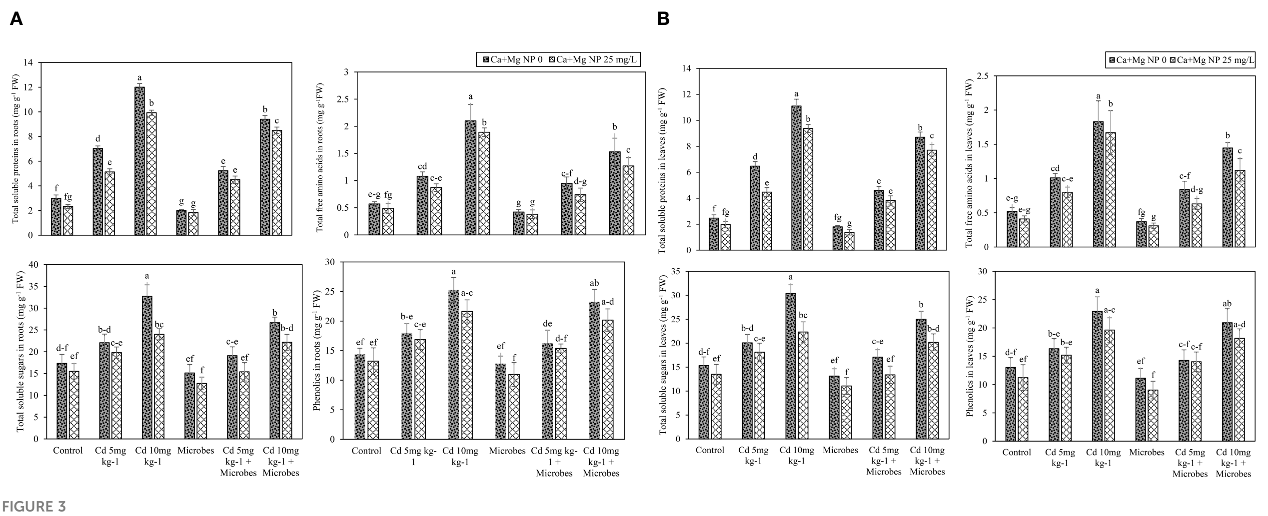


FIGURE 3
**(A)** Alone and combined effect of Ca+Mg nanocomposite (25 mg $L^{-1}$) and inoculation of *Bacillus pumilus* on total soluble proteins, total free amino acids, total soluble sugars and phenolics in roots and small letter showed the difference in significance at $p \leq 0.05$ level with mean of three replications. **(B)** Alone and combined effect of Ca+Mg nanocomposite (25 mg $L^{-1}$) and inoculation of *Bacillus pumilus* on total soluble proteins, total free amino acids, total soluble sugars and phenolics in leaves and small letter showed the difference in significance at $p \leq 0.05$ level with mean of three replications.

leaves by 3%, 5%, 4%, and 7% with Cd (5 mg $kg^{-1}$) contamination compared with Cd (5 mg $kg^{-1}$) + *B. pumilus* treatment. Similarly, Ca + Mg nanocomposite boosted the TSP, TFAA, TSS, and phenolics in roots by 2%, 23%, 11%, and 7% and leaves by 4%, 31%, 3%, and 3% with Cd (10 mg $kg^{-1}$) contamination compared with Cd (10 mg $kg^{-1}$) + *B. pumilus* treatment.

The highest increase was observed with combined Ca + Mg nanocomposite and *B. pumilus* inoculation. Further, the combined Ca + Mg nanocomposite and *B. pumilus* inoculation decreased ($p \leq 0.05$) the TSP, TFAA, TSS, and phenolics in roots by 8%, 9%, 16%, and 14% and leaves by 24%, 15%, 16%, and 19% compared with *B. pumilus* treatment. Meanwhile, the combined Ca + Mg nanocomposite and *B. pumilus* inoculation inhibited the TSP, TFAA, TSS, and phenolics in roots by 14%, 15%, 18%, and 6% and leaves by 14%, 21%, 19%, and 2% with Cd (5 mg $kg^{-1}$) toxicity compared with Cd (5 mg $kg^{-1}$) + *B. pumilus* treatment. Similarly, the combined Ca + Mg nanocomposite and *B. pumilus* inoculation also decreased the TSP, TFAA, TSS, and phenolics in roots by 10%, 17%, 8%, and 7% and leaves by 11%, 12%, 10%, and 7% with Cd (10 mg $kg^{-1}$) toxicity, compared to Cd (10 mg $kg^{-1}$) + *B. pumilus* treatment.

## 3.5 Effect of nanocomposite (Ca + Mg) and *B. pumilus* on nutrient profile

The results showed that the soil spiking with Cd (5 mg $kg^{-1}$, 10 mg $kg^{-1}$) decreased the macro and micronutrients in shoots and grains of rice over the control (Table 1). Meanwhile, Ca + Mg nanocomposite and microbial inoculation significantly increased the macro and micronutrients under Cd (5 mg $kg^{-1}$, 10 mg $kg^{-1}$) toxicity. Results revealed that the Cd (5 mg $kg^{-1}$) diminished Zn, Fe, Mg, Mn, K, and Ca in shoots by 34%, 44%, 22%, 38%, 20%, and 13% and grains by 28%, 36%, 24%, 34%, 37%, and 14% compared with control treatment. Similarly, Cd (10 mg $kg^{-1}$) at a significant ($p \leq 0.05$) lessened the concentration of Zn, Fe, Mg, Mn, K, and Ca in shoots by 65%, 82%, 38%, 70%, 35%, and 24%, and grains by 64%, 77%, 59%, 69%, 63%, and 24% compared with control treatment. Meanwhile, *B. pumilus* inoculation at a significant ($p \leq 0.05$) increased the concentration of Zn, Fe, Mg, Mn, K, and Ca in shoots by 27%, 33%, 26%, 18%, 24%, and 9% and grains by 30%, 36%, 22%, 16%, 19%, and 8% as compared with control treatment. Moreover, the *B. pumilus* inoculation increased the values of Zn, Fe, Mg, Mn, K, and Ca in shoots by 60%, 88%, 42%, 57%, 38%, and 14%, and grains by 53%, 64%, 32%, 46%, 35%, and 14% with Cd (5 mg $kg^{-1}$) contamination compared without *B. pumilus*. Under Cd (10 mg $kg^{-1}$) contamination, *B. pumilus* inoculation at a significant ($p \leq 0.05$) increased the concentration of Zn, Fe, Mg, Mn, K, and Ca in shoots by 146%, 358%, 41%, 158%, 37%, and 16% and grains by 152%, 217%, 82%, 115%, 60%, and 15% compared without *B. pumilus*.

The foliar application of Ca + Mg nanocomposite increased Zn, Fe, Mg, Mn, K, and Ca contents in shoots by 15%, 18%, 32%, 7%, 29%, and 14%, and grains by 7%, 19%, 34%, 12%, 11%, and 14% compared with control treatment. Ca + Mg nanocomposite decreased the concentration of Zn, Fe, and Mn in shoots by 19%, 25%, and 21% and increased Mg, K, and Ca 4%, 3%, and 6% with Cd (5 mg $kg^{-1}$) contamination compared with Cd (5 mg $kg^{-1}$) + *B. pumilus* treatment. Meanwhile, foliar application of Ca + Mg nanocomposite decreased the concentration of Zn, Fe, Mn, and K in grains by 30%, 24%, 13%, and 16% and increased Mg and Ca by 10% and 6% with Cd (5 mg $kg^{-1}$) contamination compared with Cd (5 mg $kg^{-1}$) + *B. pumilus* treatment. Similarly, Ca + Mg nanocomposite decreased the concentration of Zn, Fe, and Mn in shoots by 38%, 50%, and 39% and increased Mg, K, and Ca by 8%, 7%, and 8% with Cd (10 mg $kg^{-1}$) contamination compared with Cd (10 mg $kg^{-1}$) + *B. pumilus* treatment. Foliar application of Ca + Mg nanocomposite decreased the concentration of Zn, Fe, Mn, and K in grains by 38%, 39%, 19$, and 9% and increased Mg and Ca by 4% and 8% with Cd (10 mg $kg^{-1}$) contamination compared with Cd (10 mg $kg^{-1}$) + *B. pumilus* treatment.

The highest increase was observed with combined Ca + Mg nanocomposite and *B. pumilus* inoculation. Further, the combined Ca + Mg nanocomposite and *B. pumilus* inoculation at a significant ($p \leq 0.05$) increased concentration of Zn, Fe, Mg, Mn, K, and Ca in shoots by 15%, 17%, 12%, 24%, 11% and 13% and grains by 13%, 20%, 21%, 20%, 13%, and 13% compared with *B. pumilus* treatment. Meanwhile, the combined Ca + Mg nanocomposite and *B. pumilus* inoculation increased the concentration of Zn, Fe, Mg, Mn, K, and Ca in shoots by 18%, 23%, 9%, 19%, 9%, and 16% and grains by 8%, 16%, 10%, 20%, 17%, and 16% with Cd (5 mg $kg^{-1}$) contamination compared with Cd (5 mg $kg^{-1}$) + *B. pumilus* treatment. Similarly, combined Ca + Mg nanocomposite and *B. pumilus* inoculation increased the concentration of Zn, Fe, Mg, Mn, K, and Ca in shoots by 19%, 24%, 15%, 19%, 13%, and 15% and grains by 7%, 30%, 8%, 35%, 26%, and 15% with Cd (10 mg $kg^{-1}$) contamination compared with Cd (10 mg $kg^{-1}$) + *B. pumilus* treatment.

## 3.6 Effect of nanocomposite (Ca + Mg) and *B. pumilus* on Cd uptake

Statistically, soil spiking with Cd (5 mg $kg^{-1}$, 10 mg $kg^{-1}$) increased Cd levels in roots, shoots, and grains of rice over the control (Figure 4). Meanwhile, Ca + Mg nanocomposite and microbial inoculation significantly decreased the Cd uptake and toxicity with Cd (5 mg $kg^{-1}$, 10 mg $kg^{-1}$) contamination. Meanwhile, *B. pumilus* inoculation at a significant ($p \leq 0.05$) declined the uptake of Cd in roots, shoots, and grains by 61%, 55%, and 60%, compared to control. Moreover, *B. pumilus* inoculation reduced the uptake of Cd in roots, shoots, and grains by 35%, 47%, and 40% with Cd (5 mg $kg^{-1}$) contamination, compared to without *B. pumilus* inoculation. Under Cd (10 mg $kg^{-1}$) contamination, *B. pumilus* inoculation at a significant ($p \leq 0.05$) lessened the uptake of Cd in roots, shoots, and grains by 26%, 27%, and 23% compared without *B. pumilus* inoculation treatment.

Ca + Mg nanocomposite decreased the uptake of Cd in roots, shoots, and grains by 31%, 55%, and 60%, compared to the control. Meanwhile, the Ca + Mg nanocomposite augmented the uptake of Cd in roots, shoots, and grains by 18%, 24%, and 19% with Cd (5

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**TABLE 1** Macronutrients and micronutrients contents in shoots and grains under different treatments.

| Treatments | Shoots | | | | | | Grains | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Micronutrients mg kg$^{-1}$ DW | | | Macronutrients mg kg$^{-1}$ DW | | | Micronutrients mg kg$^{-1}$ DW | | | Macronutrients mg kg$^{-1}$ DW | | |
| | Zn | Fe | Mn | Ca | Mg | K | Zn | Fe | Mn | Ca | Mg | K |
| *Control* | 48.67 ± 1.38d | 38.90 ± 149d | 50.60 ± 1.51de | 231.83 ± 5.35de | 2078.80 ± 61.79f | 2273.83 ± 85.08f | 24.10 ± 1.3de | 19.60 ± 0.70d | 25.53 ± 0.70c | 116.17 ± 2.86de | 1128.73 ± 40.27d | 1241.13 ± 89.11c |
| *Cd (5 mg kg$^{-1}$)* | 32.07 ± 1.85f | 21.97 ± 1.70f | 31.50 ± 3.72g | 199.97 ± 6.89f | 1622.30 ± 82.72h | 1812.07 ± 90.43h | 17.40 ± 0.79f | 12.50 ± 0.75f | 16.80 ± 0.90f | 99.97 ± 3.13f | 865.63 ± 80.47e | 777.90 ± 60.58efg |
| *Cd (10 mg kg$^{-1}$)* | 16.90 ± 1.51h | 6.90 ± 1.51h | 15.23 ± 1.62i | 176.33 ± 4.37g | 1290.20 ± 83.43i | 1475.20 ± 59.23i | 8.57 ± 0.80h | 4.47 ± 0.86h | 7.73 ± 0.80h | 88.67 ± 2.31g | 456.87 ± 55.62f | 458.87 ± 45.51h |
| *Microbes* | 61.60 ± 1.08b | 51.60 ± 1.08b | 59.50 ± 1.15b | 251.63 ± 5.46bc | 2623.23 ± 54.78bc | 2824.53 ± 45.07bc | 31.30 ± 0.75b | 26.73 ± 0.97b | 29.63 ± 0.80b | 125.80 ± 2.45bc | 1374.90 ± 44.40bc | 1471.87 ± 46.76b |
| *Cd (5 mg kg$^{-1}$) + Microbes* | 51.40 ± 0.91d | 41.40 ± 0.91d | 49.43 ± 0.75de | 227.23 ± 5.40de | 2304.43 ± 61.25e | 2504.43 ± 61.25e | 26.60 ± 0.75cd | 20.57 ± 0.96d | 24.53 ± 0.60cd | 114.13 ± 2.41de | 1134.87 ± 41.30d | 1054.10 ± 60.21d |
| *Cd (10 mg kg$^{-1}$) + Microbes* | 41.67 ± 1.25e | 31.67 ± 1.49e | 39.43 ± 1.05f | 203.77 ± 3.72f | 1826.23 ± 62.25g | 2026.13 ± 62.25g | 21.63 ± 0.80e | 14.17 ± 0.75ef | 16.67 ± 0.87f | 101.77 ± 1.76f | 830.23 ± 55.59e | 735.20 ± 51.35fg |
| *(Ca + Mg) nanocomposite (25 mg L$^{-1}$)* | 55.77 ± 1.17c | 45.77 ± 1.17c | 53.90 ± 1.27cd | 264.57 ± 6.03b | 2734.03 ± 54.28ab | 2934.03 ± 54.28ab | 25.77 ± 0.45d | 23.40 ± 0.98c | 28.53 ± 1.12b | 132.57 ± 3.39b | 1516.03 ± 68.13ab | 1371.53 ± 46.65bc |
| *Cd (5 mg kg$^{-1}$) + (Ca + Mg) nanocomposite (25 mg L$^{-1}$)* | 41.17 ± 1.49e | 31.17 ± 1.49e | 39.20 ± 1.22f | 241.97 ± 3.44cd | 2386.30 ± 73.60de | 2586.30 ± 73.60de | 18.70 ± 0.95f | 15.53 ± 0.70e | 21.43 ± 0.65e | 120.97 ± 1.51cd | 1253.03 ± 82.02cd | 887.97 ± 26.80ef |
| *Cd (10 mg kg$^{-1}$) +(Ca + Mg) nanocomposite (25 mg L$^{-1}$)* | 25.97 ± 1.15g | 15.97 ± 1.15g | 23.87 ± 1.15h | 219.57 ± 3.91e | 1980.60 ± 84.30fg | 2180.60 ± 84.30fg | 13.47 ± 0.87g | 8.70 ± 0.55g | 13.57 ± 0.65g | 109.90 ± 1.83e | 867.27 ± 48.24e | 667.30 ± 52.86g |
| *Microbes + (Ca + Mg) nanocomposite (25 mg L$^{-1}$)* | 70.57 ± 1.72a | 60.57 ± 1.72a | 68.57 ± 1.80a | 283.90 ± 4.88a | 2932.10 ± 43.93a | 3132.10 ± 43.93a | 35.43 ± 1.07a | 32.13 ± 0.75a | 35.57 ± 1.15a | 141.90 ± 2.26a | 1665.50 ± 64.16a | 1662.10 ± 59.80a |
| *Cd (5 mg kg$^{-1}$) + Microbes + (Ca + Mg) nanocomposite (25 mg L$^{-1}$)* | 60.73 ± 1.60b | 50.73 ± 1.60b | 58.73 ± 1.40bc | 263.90 ± 5.33b | 2519.53 ± 84.92cd | 2719.53 ± 85.06cd | 28.60 ± 0.98c | 23.83 ± 0.87c | 29.50 ± 0.75b | 131.90 ± 2.72b | 1252.87 ± 112.70cd | 1231.10 ± 54.11c |
| *Cd (10 mg kg$^{-1}$) + Microbes + (Ca + Mg) nanocomposite (25 mg L$^{-1}$)* | 49.37 ± 1.56d | 39.37 ± 1.56d | 47.10 ± 1.25e | 233.60 ± 5de | 2093.60 ± 55.02f | 2293.57 ± 54.87f | 23.07 ± 0.76e | 18.47 ± 0.65d | 22.43 ± 0.86de | 116.60 ± 2.40de | 893.23 ± 54.51e | 924.80 ± 55.51de |

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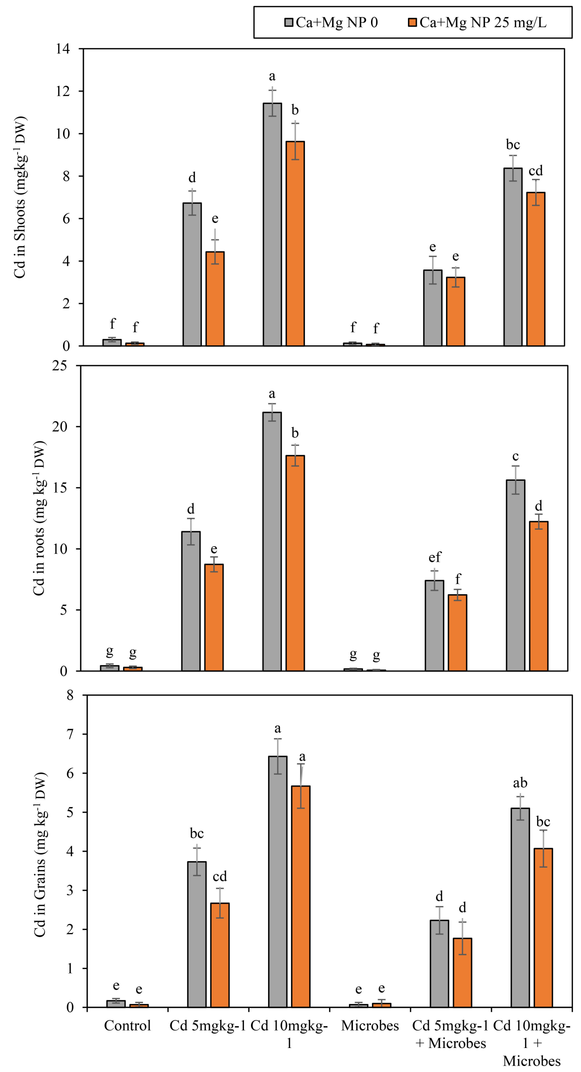


FIGURE 4
Alone and combined effect of Ca+Mg nanocomposite (25 mg $L^{-1}$) and inoculation of *Bacillus pumilus* on Cd uptake and accumulation in roots, shoots, grains, and small letter showed the difference in significance at $p \leq 0.05$ level with mean of three replications.

mg $kg^{-1}$) contamination compared with Cd (5 mg $kg^{-1}$) + *B. pumilus* treatment. Similarly, Ca + Mg nanocomposite increased Cd uptake in roots, shoots, and grains by 13%, 15%, and 11% with Cd (10 mg $kg^{-1}$) contamination, compared to Cd (10 mg $kg^{-1}$) + *B. pumilus* treatment. The highest decrease was observed with combined Ca + Mg nanocomposite and *B. pumilus* inoculation. Further, the combined Ca + Mg nanocomposite and *B. pumilus* inoculation at a significant ($p \leq 0.05$) minimized the uptake of Cd in roots, shoots, and grains by 60%, 50%, and 33% as compared with *B. pumilus* treatment. Meanwhile, the combined Ca + Mg nanocomposite and *B. pumilus* inoculation decreased the uptake of Cd in roots, shoots, and grains by 15%, 9%, and 21% with Cd (5 mg $kg^{-1}$) contamination compared with Cd (5 mg $kg^{-1}$) + *B. pumilus* treatment. Similarly, combined Ca + Mg nanocomposite and *B. pumilus* inoculation reduced the uptake of Cd in roots, shoots, and grains by 22%, 13%, and 20% with Cd (10 mg $kg^{-1}$) contamination compared with Cd (10 mg $kg^{-1}$) + *B. pumilus* treatment.

## 4 Discussion

Several studies showed that the application of exogenic CaO and MgO NPs alleviates Cd toxicity in different crops (Li et al., 2022; Nazir et al., 2022). Similarly, *B. pumilus* strains increased plant growth by decreasing Cd contamination in soil (Shahzad et al., 2021; Maslennikova et al., 2023). However, there is a gap in research regarding the combined effect of Ca–Mg nanocomposite and microbes on plants and their mitigation mechanism. Therefore, the present study examined the combined effect of Ca–Mg and *B. pumilus* inoculation on physiology, photosynthetic pigments, oxidative stress, Cd uptake, and accumulation in rice.

After Cd uptake, the high excitation energy of thylakoid situated photosynthetic electron transport was produced, which ultimately promotes ROS synthesis (MDA, $H_2O_2$, and EL) (Huihui et al., 2020). Cd also reduced the production of SOD, POD, CAT, and APX, which may hinder excessive scavenging of oxidative stress species. MgO NPs enhanced light absorption, photosynthetic function, photosystem II (PSII) efficacy, Fv/Fm, and the effective quantum yield of PSII photochemistry (FPSII). Magnesium also benefits net $CO_2$ absorption in several plant species and mitigates heavy metal stress (Tränkner et al., 2016; Samborska et al., 2018; Faizan et al., 2021). To minimize the toxic effects in heavy-metal-stressed plants, usually caused by the generation of reactive oxygen species (ROS), antioxidative protective mechanisms are activated, including the antioxidant enzymes CAT, POD, and SOD (Ahmad et al., 2018). Similarly, CaO NPs enhanced growth, antioxidative enzymes, and nutrient profile by inhibiting Cd uptake and toxicity in barley seedlings (Nazir et al., 2022). *B. pumilus* produces organic acids that bind C and solubilize phosphorus and other nutrients, which help plant growth (Sharma et al., 2013). The proposed mechanism of the Ca + Mg nanocomposite and *B. pumilus* strain is shown in Figure 5. Roots are exposed directly to the soil, and the Casparian strip might be an effective barrier to decrease Cd uptake and translocation due to the sole structure of the endothelial layer (Wu et al., 2018; Guo et al., 2021). Earlier findings discovered that Cd content in rice roots was 10 times higher than in upground plant parts, and a major portion of Cd accumulated in the cell wall, demonstrating that the root cell wall efficiently reduced Cd translocation to shoots and leaves (Liu et al., 2016; Yu et al., 2020). Cd concentration was significantly minimized with Ca, signifying an intervention between $Ca^{2+}$ and $Cd^{2+}$ ions. Cd is a nonessential and toxic element for rice plants, and Ca transporters enter the cells due to the chemical similarity (Tian et al., 2016; Ye et al., 2020). Through specific translocation channels, $Ca^{2+}$ ions impede the uptake and translocation of $Cd^{2+}$ ions by the roots, subsequently reducing the Cd concentration in plant parts (Kanu et al., 2019). Ca contents in the cytoplasm increased significantly, and signals were conveyed quickly among cells, allowing plants to mitigate Cd toxicity (Guo et al., 2018). Cd toxicity increased rice's amino groups, hydroxyl groups, cellulose, epoxide, and reactive oxygen species richness, causing structural damage to the plasma membrane and cell wall. At the same time,

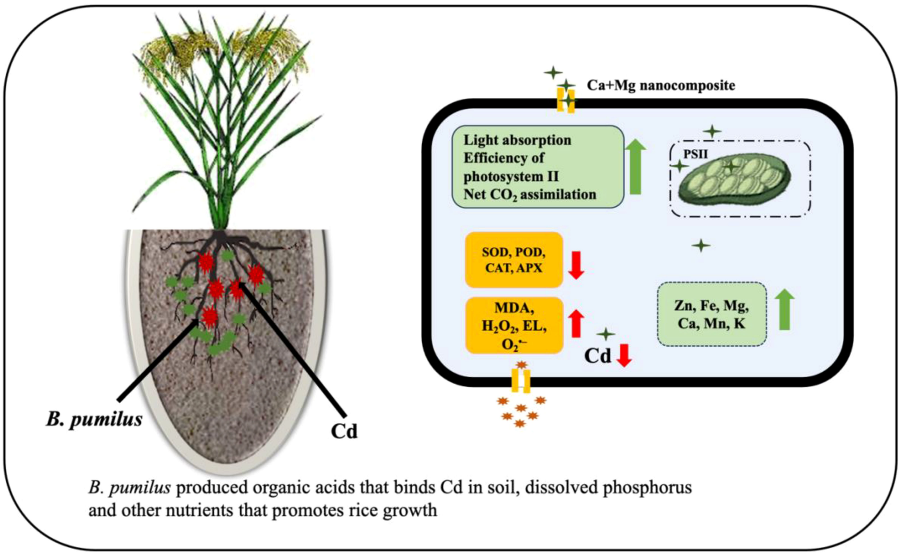


FIGURE 5
Proposed mechanism of Ca + Mg nanocomposite and *B. pumilus* to mitigate Cd toxicity and enhance rice growth and yield.

Ca minimized these unfavorable effects (Ye et al., 2020). Previous analyses indicated that Cd toxicity caused structural, chlorophyll alterations, primarily by replacing $Mg^{2+}$ ions, leading to the breakdown of chlorophyll fragments (Kanu et al., 2019). Similarly, Mg concentrations correlate negatively with Cd concentrations in rice leaves and shoots (Khaliq et al., 2019).

Cadmium is a non-essential element for plant growth with no biological function and inhibits the biomass of various plant species, including root and shoot length, dry weight, and SPAD values (Jia et al., 2020; Yang et al., 2020; Li et al., 2021). Similar results were found in the current study: Cd toxicity decreased rice plant growth parameters, including root and shoot length, root and shoot fresh and dry weight, number of tillers, and spike length, as shown in Table 2. Several studies have demonstrated that the exogenous application of NPs can alleviate Cd toxicity in wheat and rice, including Fe, Cu, $TiO_2$, and ZnO NPs (Hussain et al., 2018; Noman et al., 2020; Irshad et al., 2021; Manzoor et al., 2021). It has been reported that CaO NPs could promote root development, growth rate, and seed yield, and supply Ca in soybean and peanut (Liu and Lal, 2014, 2015). Ca and Mg are beneficial elements and enhance the plant growth. A previous study reported that the application of Ca and Mg improved growth by reducing Cd accumulation and translocation in rice (Li et al., 2022). Similarly, Mg input to chloroplast significantly enhanced the photosynthesis in rice (Li et al., 2020). Several microbes are well-known for detoxifying, transferring, and accumulating heavy metals (Pathania and Srivastava, 2020). A previous study reported that seed inoculation with *B. pumilus* increased maize growth by decreasing Cd toxicity (Shahzad et al., 2021). Fatemi et al. (2020) reported that the combined application of lead (Pb)-resistant microbes and silicon NPs improved the growth of coriander (*Coriandrum sativum* L.) under Pb stress. Previous studies showed that applying silica NPs with two microbial strains (*Azotobacter chroococcum* and *Pseudomonas koreensis*) improved the physiology and SPAD values of barely under saline stress (Alharbi et al., 2022). Our study also showed similar results: the combined application of Ca–Mg nanocomposite and *B. pumilus* inoculation minimizes Cd toxicity and increases plant growth factors (Table 3).

The literature confirmed that photosynthetic pigments are considered fundamental markers of heavy metals-induced oxidative stress (Rizwan et al., 2018). Previous studies demonstrated that CaO NPs increased chlorophyll content and gas exchange characteristics by reducing arsenic toxicity in barley. Ilyas et al. (2022) stated that seed inoculation with *Bacillus* sp. strains increased the chlorophyll contents in wheat plants. Moreover, chlorophyll content and gas exchange attributes significantly increased with the combined application of Ca + Mg and microbial inoculation. Previous studies also agreed that the combined application of ZnO NPs and Cr-resistant microbes increased chlorophyll content and gas exchange characteristics in wheat (Ahmad et al., 2022). The present study demonstrated similar findings: Ca + Mg enhanced chlorophyll and gas exchange characteristics in rice leaves under Cd contamination (Table 3).

Antioxidant enzymes activity was reduced with increasing Cd concentration (Shi et al., 2020). Cd toxicity inhibits growth and triggers oxidative damage due to the release of ROS (Marques et al., 2019). ROS damage biomolecules, proteins, carbohydrates, and lipids, particularly in membranes, harming the integral membrane and ultimately leading to cell death (Dar et al., 2017). Researchers found that Cd contamination increased MDA, $H_2O_2$, EL, and $O_2^{\bullet-}$ due to ROS generation (Du et al., 2020; Kaya et al., 2020; Ali et al., 2022a). Similar outcomes were found in our study: Cd contamination decreased antioxidant enzyme activity and increased oxidative damage by producing ROS. Similarly, the combined application of *Staphylococcus aureus* and ZnO NPs reduced ROS production and increased antioxidant enzyme activities in wheat under Cr-contaminated soil (Ahmad et al., 2022). Our study showed similar results: the combined application of Ca–Mg and *B. pumilus* inoculation

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TABLE 2 Growth parameters under different treatments.

| Treatments | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | RL | SL | RFW | SFW | RDW | SDW | NoT | NoG | SpL |
| *Control* | 10 ± 0.41cd | 70 ± 5.21cd | 15 ± .56b | 103 ± 1.80cd | 2 ± 0.06bc | 40 ± 1.74cd | 10 ± 0.77bcd | 30 ± 0.66d | 12 ± 1.13cd |
| *Cd (5 mg kg$^{-1}$)* | 8 ± 0.36f | 49 ± 2.95fg | 12 ± 0.75cde | 89 ± 2.38ef | 1 ± 0.03ef | 33 ± 1.64ef | 6 ± 0.77ef | 24 ± 1.31e | 10 ± 1.02def |
| *Cd (10 mg kg$^{-1}$)* | 6 ± 0.44h | 38 ± 5.05g | 9 ± 0.61e | 70 ± 4.27g | 1 ± 0.04g | 25 ± 1.64g | 4 ± 1.18f | 19 ± 0.89f | 7 ± 1.26f |
| *Microbes* | 13 ± 0.36b | 85 ± 2.63ab | 19 ± 1.69a | 113 ± 2.65b | 2 ± 0.078a | 47 ± 1.72ab | 12 ± 1.18ab | 34 ± 0.86bc | 14 ± 0.75bc |
| *Cd (5 mg kg$^{-1}$) + Microbes* | 9 ± 0.41ef | 54 ± 2.70ef | 13 ± 0.92bcd | 98 ± 1.81cd | 1 ± 0.12cde | 37 ± 2.30cdef | 9 ± 1.18bcde | 28 ± 1.31d | 11 ± 0.76cde |
| *Cd (10 mg kg$^{-1}$) + Microbes* | 6 ± 0.73gh | 45 ± 3.72fg | 10 ± 0.88de | 84 ± 3.35f | 1 ± 0.04fg | 32 ± 2.12f | 7 ± 1.18def | 24 ± 1.08e | 9 ± 1.09ef |
| *(Ca + Mg) nanocomposite (25 mg L$^{-1}$)* | 13 ± 0.61b | 79 ± 3.13bc | 18 ± 0.55a | 113 ± 3.98b | 2 ± 0.06a | 46 ± 2.35ab | 12 ± 1.18abc | 36 ± 1.08b | 16 ± 0.83ab |
| *Cd (5 mg kg$^{-1}$) + (Ca + Mg) nanocomposite (25 mg L$^{-1}$)* | 10 ± 0.35cde | 63 ± 2.30de | 14 ± 1.17bc | 98 ± 3.16cd | 1 ± 0.03bcd | 38 ± 1.56cde | 9 ± 1.18cde | 29 ± 0.43d | 12 ± 1.10cde |
| *Cd (10 mg kg$^{-1}$) +(Ca + Mg) nanocomposite (25 mg L$^{-1}$)* | 8 ± 0.32fg | 53 ± 4.21ef | 11 ± 0.35cde | 82 ± 3.27f | 1 ± 0.04def | 33 ± 1.60ef | 7 ± 1.18ef | 24 ± 0.43e | 10 ± 0.60ef |
| *Microbes + (Ca + Mg) nanocomposite (25 mg L$^{-1}$)* | 15 ± 0.76a | 92 ± 4.93a | 21 ± 1.42a | 124 ± 3.21a | 2 ± 0.08a | 51 ± 1.93a | 15 ± 0.77a | 39 ± 0.66a | 17 ± 0.88a |
| *Cd (5 mg kg$^{-1}$) + Microbes + (Ca + Mg) nanocomposite (25 mg L$^{-1}$)* | 11 ± 0.80bc | 70 ± 3.58cd | 15 ± 1.16b | 107 ± 1.23bc | 2 ± 0.07b | 42 ± 2.74bc | 10 ± 1.18bc | 33 ± 0.86c | 14 ± 0.83bc |
| *Cd (10 mg kg$^{-1}$) + Microbes + (Ca + Mg) nanocomposite (25 mg L$^{-1}$)* | 9 ± 0.61def | 60 ± 3.18de | 12 ± 1.15bcd | 97 ± 3.20de | 1 ± 0.10de | 36 ± 2.02f | 9 ± 1.18bcde | 29 ± 1.31d | 12 ± 1.20cde |

TABLE 3 Photosynthetic and gas exchange parameters under different treatments.

| Treatments | SPAD | Chl a | Chl b | Total chl | Carotenoi-ds | Pn | Tr | WUE | SC |
|---|---|---|---|---|---|---|---|---|---|
| *Control* | 40 ± 2.40cde | 3 ± 0.20bcd | 2 ± 0.06bcd | 5 ± 0.25bcd | 1.2 ± 0.10bc | 10 ± 0.30bc | 3 ± 0.15bc | 7 ± 0.30cd | 2 ± 0.12cd |
| *Cd (5 mg $kg^{-1}$)* | 35 ± 3.09def | 2 ± 0.20def | 1 ± 0.10f | 4 ± 0.25efg | 0.8 ± 0.10def | 8 ± 0.40de | 2 ± 0.20cde | 5 ± 0.15fg | 1 ± 0.14ef |
| *Cd (10 mg $kg^{-1}$)* | 26 ± 3.03g | 2 ± 0.25f | 1 ± 0.10g | 3 ± 0.20g | 0.5 ± 0.05f | 6 ± 0.50f | 2 ± 0.20e | 4 ± 0.40h | 1 ± 0.10f |
| *Microbes* | 49 ± 2.94ab | 3 ± 0.30ab | 2 ± 0.10b | 6 ± 0.46b | 1.4 ± 0.10ab | 12 ± 0.60b | 3 ± 0.20a | 8 ± 0.40ab | 2 ± 0.14ab |
| *Cd (5 mg $kg^{-1}$) + Microbes* | 38 ± 2.25cdef | 3 ± 0.31cde | 2 ± 0.10de | 4 ± 0.31cde | 1 ± 0.10cd | 10 ± 0.50c | 2 ± 0.15bcd | 6 ± 0.60def | 2 ± 0.14de |
| *Cd (10 mg $kg^{-1}$) + Microbes* | 31 ± 2.59fg | 2 ± 0.20ef | 1 ± 0.10f | 4 ± 0.31fg | 0.8 ± 0.10def | 7 ± 0.60e | 2 ± 0.20de | 5 ± 0.35g | 1 ± 0.07ef |
| *(Ca + Mg) nanocomposite (25 mg $L^{-1}$)* | 44 ± 3.13bc | 3 ± 0.20abc | 2 ± 0.10bc | 5 ± 0.35bc | 1.3 ± 0.10ab | 11 ± 0.40b | 3 ± 0.20ab | 7 ± 0.30bc | 2 ± 0.12bc |
| *Cd (5 mg $kg^{-1}$) + (Ca + Mg) nanocomposite (25 mg $L^{-1}$)* | 40 ± 2.39cde | 3 ± 0.26bcde | 2 ± 0.06de | 5 ± 0.32def | 0.9 ± 0.10de | 10 ± 0.40cd | 3 ± 0.20bcd | 6 ± 0.35cde | 2 ± 0.09cd |
| *Cd (10 mg $kg^{-1}$) +(Ca + Mg) nanocomposite (25 mg $L^{-1}$)* | 34 ± 2.51efg | 2 ± 0.25def | 1 ± 0.10ef | 4 ± 0.31efg | 0.7 ± 0.10ef | 8 ± 0.45e | 2 ± 0.25de | 5 ± 0.30efg | 1 ± 0.0.08ef |
| *Microbes + (Ca + Mg) nanocomposite (25 mg $L^{-1}$)* | 54 ± 2.55a | 4 ± 0.20a | 2 ± 0.10a | 7 ± 0.30a | 1.5 ± 0.10a | 14 ± 0.50a | 4 ± 0.26a | 9 ± 0.50a | 3 ± 0.14a |
| *Cd (5 mg $kg^{-1}$) + Microbes + (Ca + Mg) nanocomposite (25 mg $L^{-1}$)* | 43 ± 1.75bcd | 3 ± 0.25bcd | 2 ± 0.15bcd | 5 ± 0.53bcd | 1.2 ± 0.10bc | 11 ± 0.31b | 3 ± 0.35bc | 7 ± 0.30bcd | 2 ± 0.19bcd |
| *Cd (10 mg $kg^{-1}$) + Microbes + (Ca + Mg) nanocomposite (25 mg $L^{-1}$)* | 36 ± 2.01def | 2 ± 0.15def | 1 ± 0.15cd | 4 ± 0.31def | 0.9 ± 0.05cde | 9 ± 0.35cd | 2 ± 0.20cde | 6 ± 0.30defg | 2 ± 0.19de |





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decreased ROS production by reducing EL%, MDA, $H_2O_2$, and $O_2^{\bullet-}$ concentrations and increased antioxidant enzyme activities by increasing POD, SOD, APX, and CAT concentrations in roots and leaves (Figures 2A, B).

Cd toxicity increased the osmolytes (proline and glycine betaine), sugar content, and secondary metabolites in mustard (*Brassica juncea*) plants (Ahmad et al., 2016). In contrast, the application of *Pseudomonas aeruginosa and Burkholderia gladioli*) decreased Cd toxicity in *Solanum lycopersicum* seedlings by modulating the expression of secondary metabolites (Khanna et al., 2019). Similar outcomes were found in our study: *B. pumilus* inoculation decreased Cd toxicity by regulating secondary metabolites. The current study showed that Cd toxicity significantly increased secondary metabolites such as TSP, TFAA, TSS, and phenolics in roots and shoots, while combined application of Ca–Mg and *B. pumilus* inoculation slightly decreased secondary metabolism in leaves and roots (Figures 3A, B).

Nano-fertilization has been considered a game-changer in addressing nutritional insecurity, especially in developing countries (Wang et al., 2020; Cao et al., 2022);. The application of CaO NPs decreased the Cd concentration in the roots, shoots, and grains of barley plants and increased the macro and micronutrients (Nazir et al., 2022). Similarly, gallic acid increased wheat growth and decreased the uptake of Cr in roots, shoots and grains under tannery wastewater stress (Nafees et al., 2024b). Previously, Ca and Mg reduced the Cd concentration in rice roots, shoots, and grains (Li et al., 2022). Similar results were found in our study: the combined application of foliar Ca–Mg and *B. pumilus* decreased the Cd concentration in rice roots, shoots, and grains (Figure 4) and increased the macro and micronutrients (Zn, Fe, Mg, Mn, K, and Ca) in shoots and grains (Table 1). Therefore, during phytoremediation, the foliar application of Ca + Mg nanocomposite and microbial (*B. pumilus*) inoculation will not only raise the market value of agricultural products but also improve the nutritional value of rice grains, which is of great significance in addressing hidden hunger.

## 5 Conclusion

The present study investigated how cadmium toxicity reduced plant growth, biomass, and gas exchange characteristics in rice. Additionally, Cd-stressed rice demonstrated increased MDA, $H_2O_2$, EL, and $O_2^{\bullet-}$ levels and higher Cd concentration. However, the application of Ca + Mg nanocomposite and *B. pumilus* individually modulated the antioxidant system of treated rice by improving the activity of CAT, SOD POD, and APX. Moreover, the combined application of Ca + Mg nanocomposite and *B. pumilus* further enhanced the growth and physiochemical features of rice seedlings grown under both standard and Cd-contaminated conditions. Further studies are required to understand the molecular mechanisms involved in Cd tolerance in different crops through the synergistic application of Ca + Mg nanocomposite and *B. pumilus*.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Author contributions

MuA: Writing – review & editing, Writing – original draft, Resources, Methodology, Formal analysis, Conceptualization. MN: Writing – review & editing, Resources, Data curation, Conceptualization. MW: Conceptualization, Resources, Writing – review & editing, Data curation. SOA: Data curation, Writing – review & editing, Formal analysis. KA-G: Investigation, Formal analysis, Writing – review & editing. MoA: Methodology, Writing – review & editing. HZ: Software, Formal analysis, Writing – review & editing. SA: Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. FL: Supervision, Resources, Project administration, Funding acquisition, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Publisher's note

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