



Calcium Nanoparticles Impregnated With Benzenedicarboxylic Acid: A New Approach to Alleviate Combined Stress of DDT and Cadmium in *Brassica alboglabra* by Modulating Bioaccumulation, Antioxidative Machinery and Osmoregulators

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Specialty section:

This article was submitted to
Plant Biotechnology,
a section of the journal
Frontiers in Plant Science

Received: 30 November 2021

Accepted: 18 January 2022

Published: 09 March 2022

Citation:

Mubeen S, Shahzadi I, Akram W,
Saeed W, Yasin NA, Ahmad A,
Shah AA, Siddiqui MH and Alamri S
(2022) Calcium Nanoparticles
Impregnated With
Benzenedicarboxylic Acid: A New
Approach to Alleviate Combined
Stress of DDT and Cadmium
in *Brassica alboglabra* by Modulating
Bioaccumulation, Antioxidative
Machinery and Osmoregulators.
Front. Plant Sci. 13:825829.
doi: 10.3389/fpls.2022.825829

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At present, the alleviation of stress caused by climate change and environmental contaminants is a crucial issue. Dichlorodiphenyltrichloroethane (DDT) is a persistent organic pollutant (POP) and an organochlorine, which causes significant health problems in humans. The stress caused by cadmium (Cd) and the toxicity of DDT have direct effects on the growth and yield of crop plants. Ultimately, the greater uptake and accumulation of DDT by edible plants affects human health by contaminating the food chain. The possible solution to this challenging situation is to limit the passive absorption of POPs into the plants. Calcium (Ca) is an essential life component mandatory for plant growth and survival. This study used impregnated Ca (Bd_{Ca}) of benzenedicarboxylic acid (Bd) to relieve abiotic stress in plants of *Brassica alboglabra*. Bd_{Ca} mitigated the deleterious effects of Cd and reduced DDT bioaccumulation. By increasing the removal efficacy (RE) up to 256.14%, Bd_{Ca} greatly decreased pollutant uptake (Cd 82.37% and DDT 93.64%) and supported photosynthetic machinery (86.22%) and antioxidant enzyme defenses (264.73%), in applied plants. Exogenously applied Bd also

successfully improved the antioxidant system and the physiochemical parameters of plants. However, impregnation with Ca further enhanced plant tolerance to stress. This novel study revealed that the combined application of Ca and Bd could effectively relieve individual and combined Cd stress and DDT toxicity in *B. alboglabra*.

Keywords: abiotic stress, bioaccumulation, *Brassica alboglabra*, cadmium uptake, DDT, heavy metal removal, organochlorine, persistent organic pollutant

INTRODUCTION

Dichlorodiphenyltrichloroethane (DDT), an organochlorine and a persistent organic pollutant (POP), was first synthesized in 1874 to control insect pests on large scales in the agriculture fields (Turusov et al., 2002). Its effectiveness evolved into the most abundantly used pesticide before it was banned by the Environmental Protection Agency (EPA) in the United States during 1972 (Jaga and Dharmani, 2003). It was famous for the long-term pest control, more effectiveness, easy manufacturing, and cost-effectiveness (Sadasivaiah et al., 2007). Besides, DDT is also released as a by-product of all the chemical industries dealing with or producing chlorinated chemicals/liquids. Because DDT has been used in bulk amounts worldwide and for long periods, it has become a high-ranked source of toxicity for the human population without any geographical discrimination. Some other chemical processes that involved chlorine (e.g., smelting of metals, bleaching of paper pulp, production of cement, impregnation of wood, and incineration of solid waste and sewage sludge) add a significant share in the DDT contents in the environment.

Cadmium (Cd) is among the most injurious metals imposing multiple detrimental effects on the growth and development of plants and concomitantly on humans (Sardar et al., 2022a). Rapid industrialization and excessive use of Cd based fungicides, herbicides, insecticides, and fertilizers are continuously contaminating soils and water (Sardar et al., 2022b). Cd-contaminated soils disturb enzymatic activities, metabolic processes, plant growth, and quality of the plant produce (Shah et al., 2021). The consumption of Cd-contaminated plant commodities may cause gastrointestinal and pulmonary disorders besides cancer. Soil Cd uptake is a primary source of food chain contamination. Cd is bioaccumulation in the foliage ($> 5\text{--}10 \mu\text{g g}^{-1}$) and has harmful effects on the physiochemical activities of plants. Henceforward, it becomes indispensable to adopt some suitable strategies to lower Cd toxicity and reduce its uptake by plants growing in polluted sites.

Both Cd and DDT are readily absorbed in plant tissues, translocated between different tissues, and preserved in the lipid-rich areas. Complete avoidance of these pollutants is necessarily important, which seems impossible if the pollutants are part of our everyday diet. Therefore, limiting Cd and DDT contents in agri-food products can greatly assist in avoiding pollutant bioaccumulation in human tissues.

Brassica is an oil seed-producing plant famous for its consumption as a leafy vegetable and animal feed. Vegetables are perishable crops, therefore, mostly cultivated in agricultural lands situated near urban areas. In such regions, crops may

be irrigated by water contaminated with industrial wastewater containing Cd and other metal contaminants (Rezapour and Samadi, 2011). Cost-effective DDT has been extensively used for the management of insect pests of several crops. Residues of DDT are still present in these soils (Mansouri et al., 2017). Growing such an important crop in a DDT- and Cd-polluted environment causes the pollutants to be absorbed, translocated, and preserved in the edible plant parts. Plant pollutants cause orchestration in the concentration of total soluble protein, soluble sugars, leaf water, and osmoprotectants besides the decline in the activity of antioxidant enzymes, including ascorbate peroxidase (APX), superoxide dismutase (SOD), and peroxidase (POD) in stressed plants (Yasin et al., 2018a; Ahmad et al., 2020b; Li et al., 2021). They severely affect plant growth, photosynthesis, chlorophyll activity, antioxidant activity, reactive oxygen species (ROS), and many other physiological attributes.

Benzenedicarboxylic acid (Bd) is well-documented for driving plant physiological responses toward a new equilibrium. It successfully induced antioxidant defenses, improved enzymatic activities, and augmented the nutritional quality of fruits (Ahmad et al., 2019). Considering its diverse physiological effects in plants, and potential to improve fruit quality, Bd was selected to inhibit DDT and Cd absorption from the surrounding environment. This is the first study that evaluates the molecule in limiting the absorption and translocation of plant stressors. Nevertheless, Bd is readily soluble in water. Therefore, it can be easily scavenged or washed off from plant surfaces during rain. Its molecules contain chromophores (with absorbance at wavelengths $>290 \text{ nm}$), therefore, stay highly susceptible to photolysis by sunlight, and also degrade readily in water (Lu et al., 2002). Consequently, its stable, slow, and continuous release needs some safeguard agent for which calcium (Ca) impregnation could be a useful strategy.

Calcium nanoparticles are a family of inorganic layered materials (Ram Reddy et al., 2006; Xu et al., 2006; Reddy et al., 2008), which can be synthesized using a cost-effective protocol (Xu et al., 2006). The main feature of Ca includes its role in the formation of plant structure. The Ca can be applied to plants for increased growth and defense parameters (Thor, 2019). Several positive physiological impacts have already been documented on Ca application (Fletcher et al., 2020). Ca alleviates Cd and other abiotic stresses in applied plants (Sakouhi et al., 2021; Tong et al., 2021).

To reduce the accumulation level of Cd and DDT in crops, to ensure food safety, and to reduce human intake, this study found an additive that can effectively reduce the absorption of pollutants by crops. It was hypothesized that the combined application of Ca with Bd might have beneficial effects on plant stress alleviation and growth improvement. According to

our information, there is no study demonstrating the role of Bd and/or Ca in alleviating multiple environmental stresses. Henceforth, this study is designed to investigate the role of Bd-coated Ca (Bd_{Ca}) in alleviating multiple stress (DDT and Cd) conditions in Kale plants and their joint effect of controlling the bioaccumulation of the toxic organic contaminant. This study elucidates the role of Bd_{Ca} on growth, gas exchange attributes, and antioxidative system of *Brassica alboglabra* plants under DDT and temperature stress. Moreover, this study was deliberated to examine the potential of Bd_{Ca} in reducing the bioaccumulation of toxic organic contaminant, i.e., DDT.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Seeds of *B. alboglabra* were surface sterilized by submerging in 0.1% potassium permanganate for 15 min and subsequent thorough washing by using deionized water. The 10 sterilized seeds were placed on 2 layers of 3-mm Whatman filter paper embedded in 9 cm glass jars supplemented with 10 ml of standard Davtyan (0.75 N) nutrient solution in ddH₂O (Daryadar et al., 2019). All aforementioned steps were performed beneath very low light. Then, these covered jars were randomly kept in a plant growth chamber in the dark at 25 ± 2°C for 5 days. After the growth of the initial 5 days, seedlings were transferred to pots (2 plants/pot) packed with 1 kg of Cd-contaminated UQ23 pot soil.

Bd_{Ca} Synthesis

The method by Mitter et al. (2017) was strictly followed to prepare Ca nanoparticles. The process included non-aqueous precipitation, heat treatment, purification step, and dispersion in water. The complete procedure yielded an average particle size of 45 nm, which were analyzed by a Nanosizer, Nano ZS instrument (Malvern Instruments) to obtain the Z average size and particle diameter (PdI). The chemical composition and crystal structure were verified by powder X-ray diffraction (XRD) with five Ca samples (Rigaku Miniflex X-Ray Diffractometer), Fourier-transform IR spectroscopy (Nicolet 6700 FTIR; Thermo Electron Corporation) with attenuated total reflection mode (Xu et al., 2006), and imaged by JEOL Transmission Electron Microscope, JSM-2010 (Supplementary Data Set 1).

Loading of Bd

To define optimal and complete loading of respective Bd into Ca nanoparticles, the ratio of Bd/Ca was adjusted to 1:10. A mixture of 10 ml of Bd and 100 ml of Ca was prepared and incubated at room temperature for 30 min with continuous agitation of 120 rpm. The quantification of the residual Bd was carried out using the standard method developed by Sun et al. (2020). High-performance liquid chromatography (HPLC) system equipped with ZORBAX-Eclipse XDB-C18 column (4.6 mm × 250 mm, 5 μm, Agilent) separated the free Bd molecule aqueous mobile phase of 40% methanol flowing at the rate of 0.4 ml min⁻¹. Samples of 10 μl were separately processed at 40°C, and absorbance readings were recorded at 210 nm. The stable release of Bd was also ensured at three different incubation temperatures

of 0°C, 25°C, and 50°C for a period of 60 by using a method as described earlier (Supplementary Data Set 1).

Treatment Application

Bd (C₁₉H₂₂O₆) was purchased from the RPI Research Products International, CAS # 77-06-5. The growth regulator was initially dissolved in ethanol to prepare a stock concentration of 0.5 mM L⁻¹ and then diluted with distilled sterilized water to attain a concentration of 5 μM L⁻¹ as a working concentration recommended by Miao et al. (2017). Similarly, the Bd_{Ca} solution was prepared to get the final working concentration of 0.5 mM L⁻¹ for Bd. However, the concentration of Ca was 1 g L⁻¹. The third treatment of Ca nanoparticles was also prepared (1 g L⁻¹). Each seedling received 0.5 ml of the respective spray treatment.

To elucidate the pathway-specific uptake of DDT into the plants, the experiment was designed to expose potted *B. alboglabra* plants to DDT through the use of incubation chambers (ICs). The ICs were designed and built using glass material by strictly following the method of a previous study (Zhu et al., 2016). There were two separate entry points for air and DDT into the chamber, powered with electric pumps and connected through polytetrafluoroethylene pipes. Chambers also have an air sampling system and a temperature regulation system. A separate chamber was used for each treatment. The potting soil was covered with a 2-cm layer of silica sand (150–380 μm) to minimize the DDT exchange between the soil and the air (Zhu et al., 2020). The DDT concentration in the air was adjusted to 1,000 mg m⁻³ as derived from its minimum toxic concentration of 582.4 mg m⁻³ and kept constant throughout the incubation period (Jarrell et al., 2002). To compensate for the loss of particulate on the plant surface over time, the POP dust-spraying step was repeated weekly. Later, the conditions of ICs were adjusted at 70–76% relative humidity and 500–550 mmol m⁻² s⁻¹ light with a 16-h photoperiod. Five replications were used for each treatment. Cd treatment was applied to the plants by strictly following the method developed by Li et al. (2021). All these dose treatments were adopted based on the previous literature and the highest quantity of pollutants recorded in the environment. Furthermore, the concentration of the pollutants was kept stable in the growth chamber.

Determination of Growth, Physiological, and Antioxidant Defense Parameters

After 8 weeks of incubation, entire plants were sampled randomly. The harvested plants were separated into the root, shoot, and leaf samples. These plant parts were kept individually in distilled water for 60 s. Later, plant parts were dried on blotting paper and stored at -80°C. The plant samples were processed for the estimation of Leaf Relative Water Content (LRWC), Photosynthetic pigments (Chl *a* and Chl *b*), Soluble Sugars, Gas Exchange Parameters, net Photosynthetic Rate (*P_n*), Estimation of Cd Contents, Metal Tolerance Index (MTI), Evaluation of Hydrogen Peroxide, Lipid Peroxidation, Electrolyte Leakage (EL), Leaf Osmotic Potential, Membrane Stability Index (MSI), Estimation of Water Potential, Proline Content, β-carotenoids

Contents, and the parameters of Oxidative and Antioxidant Enzymatic Activities using the standard optimized protocols (Khan et al., 2019).

Quantification of DDT

The DDT was quantified by gas chromatography-mass spectrometry (GC-MS), as described by Baumer et al. (2020). The system consisted of GC-MS (QP) by Shimadzu (Kyoto, Japan), coupled with a mass detector and a capillary column. Detailed specifications were 30 m DB-5, 0.1 μm film thickness, and 0.25 mm internal diameter of the capillary column used for the determination of DDT. Samples were injected using a splitless injection mode while keeping an ion source temperature of 250°C and using helium as the carrier gas at 0.75 ml/min flow rate. The oven temperature was ramped at the rate of 4°C min⁻¹, initializing its range from 120°C to 190°C, and at the rate of 32°C min⁻¹ up to 270°C, which was kept constant for 4 min. By operating the mass spectrometer at scanning mode between 45 and 475 Da (m/z), DDT was detected in the sample size of 50 μl with a minimum limit of 1 mg kg⁻¹.

Calculation of Mobility, Distribution, and Remediation Efficiency

The translocation factor (TF) and avoidance efficiency (AE) were used to evaluate the ability of the plants to absorb, translocate, and/or avoid pollutants, according to the following equations (Xiao et al., 2019; Zeng et al., 2019):

$$(1) \text{ TF} = C_{\text{substance shoot}}/C_{\text{substance leaf}}$$

$$(2) \text{ AE} = (C_{\text{substance shoot}} \times M_{\text{shoot}} \times C_{\text{substance leaf}} \times M_{\text{leaf}}) / (C_{\text{substance air}} \times M_{\text{air}})$$

where $C_{\text{substance shoot}}$, $C_{\text{substance leaf}}$, and $C_{\text{substance air}}$ are the contents of DDT (mg·kg⁻¹) in shoots, roots, and air, respectively. However, the mass of one cubic meter of air was standardized as 1.29 kg during the experimentation.

Statistical Analysis

Randomized one-way ANOVA was used for the comparisons between different treatments. Means were compared using the all pairs Tukey's honestly significant difference test. Statistical calculations of ANOVA were performed using DSAASTAT statistics software.

RESULTS

Evaluation of Bd_{Ca}

Transmission electron micrograph revealed a single crystalline Ca (44 μm) impregnated with Bd. The comparison of the three FTIR spectra (i.e., Bd, Ca, and Bd_{Ca}) had one unique dejection at 2,237 in Bd_{Ca}, which was absent in Bd and Ca, showing a stable bond between them. The XRD assay came up with the two characteristic peaks in Bd, one in Ca, and three in Bd_{Ca}, showing the sustained identity of the two impregnated substances (Figure 1).

Determination of Growth Attributes

The results of this study showed that Bd alone had the potential to boost the growth parameters of the plants, i.e., root fresh weight, shoot fresh weight, root dry weight, and shoot dry weight under multiple stress conditions of DDT toxicity and Cd stress. All these growth credentials were improved 1.3–1.9 times after Bd treatment. However, another interesting finding was the positive support of C toward the plant growth parameters. Growth parameters were elevated by 1.3–1.6 times due to Ca use. The growth regulator Bd allowed *Brassica* plants to overcome DDT toxicity, introducing pollutant tolerance in them. Besides, it also elevated the plant growth parameters up to 162% (Supplementary Figure 1). However, Ca could enhance the plant growth, up to 131%, when exposed to DDT. The maximum growth enhancement (258%) was reported in the plants exposed to Bd_{Ca}, and it could be reduced up to 221% under DDT toxicity. In contrast, the toxic effects of DDT caused a 34–49% reduction in growth attributes and leaf area of the plants as opposed to control therapy. Bd, Ca, and Bd_{Ca} were proved successful in augmenting chlorophyll contents in *Brassica* plants. However, enhancement in chlorophyll contents was recorded as 139% by Bd application, 125% by Ca procedure, and 158% by Bd_{Ca}. DDT stress plants also displayed a substantial increase of 147% in chlorophyll contents when primed with Bd_{Ca}. Cd toxicity exerts deleterious effects on *Brassica* plants, retarding 18% of growth attributes, 21% of leaf area, and 15% chlorophyll contents (Supplementary Figure 2).

Estimation of Leaf Relative Water Content

The findings showed the impact of Cd and DDT on LRWC (53.24 and 18.61%, respectively) relative to the *Brassica* plant. The maximum LRWC was recorded in the negative Cd control treatments. However, as opposed to the control, the Bd treatment increased the value of LRWC (46.31%). Ca application to *Brassica* plants also increased LRWC by 36.98%, while under Bd_{Ca} treatment, a 51.76% increase in LRWC was evident. The treatment with DDT reduced P_n to 51.37%. A significant increase in P_n due to Bd and Ca was found at 25.61% and 22.13%, respectively. The Bd_{Ca} application reported a maximum P_n increase of 40.25%. As far as the photosystem-quenching Fv/Fm is concerned, the effect of Bd-impregnated Ca treatment was significantly higher than the individual effects of Ca and Bd (28.66%). Bd_{Ca} was the most successful plant treatment that produced the greatest improvement in stomatal conductance, C_i , and the rate of transpiration. However, all these physiological parameters were negatively affected by the stress caused by Cd and DDT toxicity. The cell physiology of *Brassica* was positively affected by both Bd and Ca (Figure 2).

Determination of Photosynthetic Pigments and Soluble Sugars

The DDT toxicity substantially decreased photosynthetic pigments by up to 46.31% as compared to the control treatment. Bd induced an elevation of 1.35 times in the pigments and Ca treatment of 1.24 times. Bd_{Ca} lied in the stronger support to the

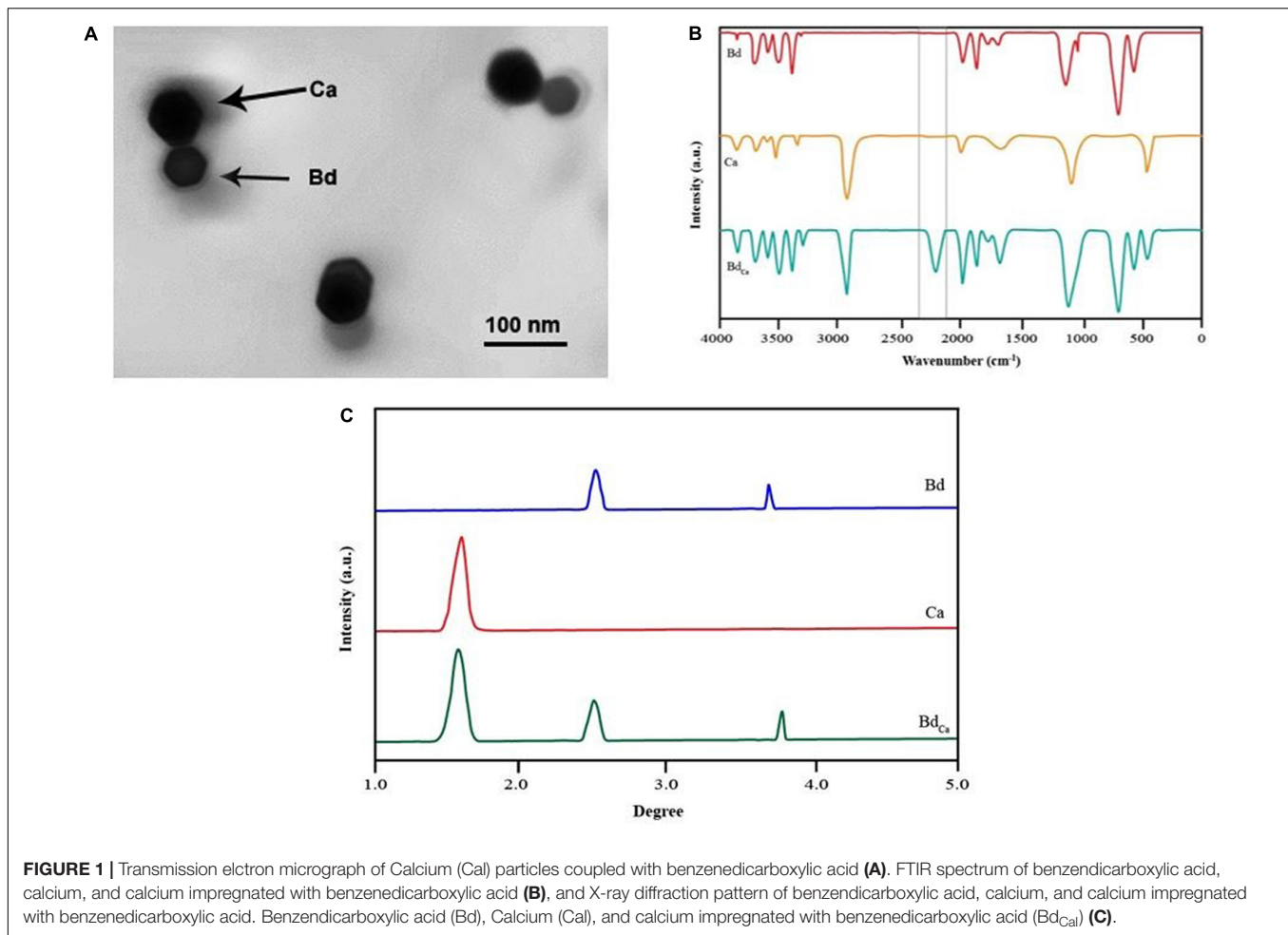


FIGURE 1 | Transmission electron micrograph of Calcium (Ca) particles coupled with benzenedicarboxylic acid (A). FTIR spectrum of benzenedicarboxylic acid, calcium, and calcium impregnated with benzenedicarboxylic acid (B), and X-ray diffraction pattern of benzenedicarboxylic acid, calcium, and calcium impregnated with benzenedicarboxylic acid. Benzenedicarboxylic acid (Bd), Calcium (Ca), and calcium impregnated with benzenedicarboxylic acid (Bd_{Ca}) (C).

overall chlorophyll contents, raising it to 235.78%. A 17.92% reduction in chlorophyll in the Kale plants was caused by Cd. DDT was responsible for a substantial decrease in the soluble sugar content of 3.18 mg/g when compared to the control treatment of 5.48 mg/g. Bd (12.06 mg/g) and Ca treatment (9.92 mg/g) recorded the substantial mitigation of the soluble sugar contents. While, in the presence of DDT, Bd_{Ca} increased the soluble sugar content up to (12.38 mg/g), whereas, in the absence of toxic DDT, the contents were increased up to 14.69 mg/g. In terms of soluble sugars, Cd stress had deleterious effects on *Brassica* plants. However, Bd_{Ca} (mg/g) could successfully mitigate the detrimental effects of the plant stressors (Table 1).

Antioxidant Defenses

Nitrate reductase activity was significantly decreased by the toxic content of DDT and increased by the application of Bd and Ca. Bd_{Ca} resulted in the most successful mitigation, which increased the activity of the enzyme by 247.64%. The enzyme activity was adversely affected by Cd, and Bd_{Ca} significantly mitigated Cd and DDT toxicity, resulting in an increase in nitrate reductase activity of 218.39%. Similar patterns have been observed for other enzymes studied, such as catalase, POD, SOD, and carbon anhydrase. DDT toxicity and Cd variations adversely affected the

activities of the enzymes. However, Bd_{Ca} effectively alleviated the hazardous effects and increased the activity of the enzymes by up to 227.36%, improving the antioxidant protection of the plant (Figure 3).

Electrolyte Leakage Malondialdehyde Content (MDA) and H₂O₂

The DDT toxicity and Cd stress boosted the EL. Due to DDT, an increase of 234.74% was reported in EL. However, in comparison with the control treatment, the increased EL due to Cd was significantly elevated. Under the toxic effects of DDT, malondialdehyde content (MDA) was also increased by more than two times. The H₂O₂ content was elevated by toxic DDT and Cd (223.54% and 208.36%, respectively) by strictly following the identical pattern. Successfully downregulated H₂O₂ contents by Bd and Ca treatments minimized the risk of oxidative harm to plant cells (Figure 3).

Enzymatic Analysis

The enzymatic content of monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione-S-transferase (GST), glutathione peroxidase (GPX), glutathione reductase (GR), and APX in Kale plants was significantly

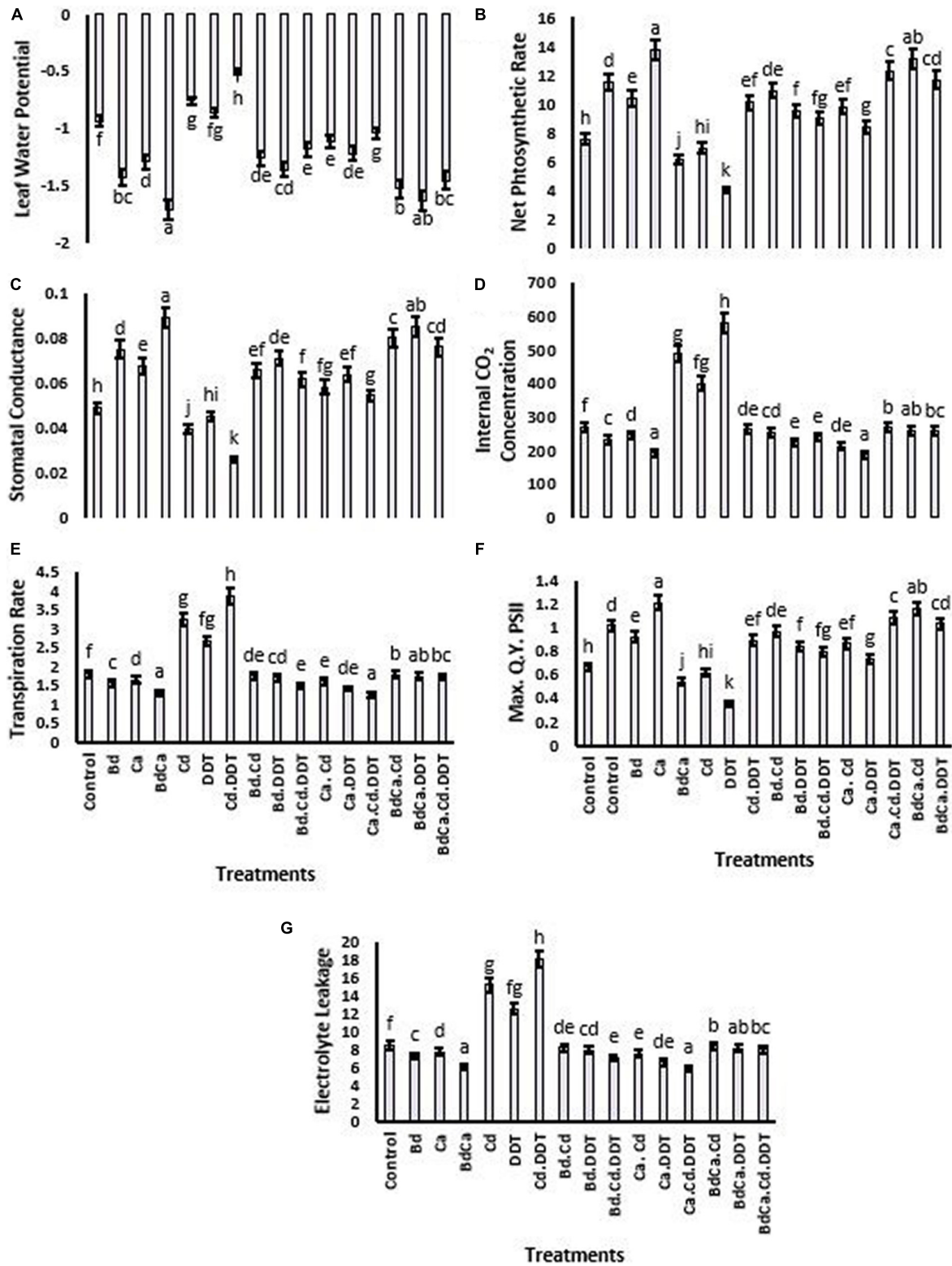


FIGURE 2 | Effect of benzenedicarboxylic acid (Bd) on photosynthesis-related parameters of *Brassica alboglabra* under toxic conditions of dichlorodiphenyltrichloroethane (DDT) and cadmium (Cd). Leaf relative water content (A), net photosynthesis rate (B), stomatal conductance (C), internal CO₂ concentration (D), transpiration rate (E), maximum quality yield of PS-II (F), and electrolyte leakage (G). Values demonstrate means ± SD (n = 5). Different letters indicate a significant difference among the treatments (P ≤ 0.05). Bd, benzenedicarboxylic acid; Cd, cadmium; DDT, dichlorodiphenyltrichloroethane; BdCa, benzenedicarboxylic acid doped calcium.

TABLE 1 | Effect of benzenedicarboxylic acid (Bd) and calcium nanoparticles (Ca) on chlorophyll *a*, chlorophyll *b*, total chlorophyll, and soluble sugars of *Brassica alboglabra* under Cd stress, temperature stress, and BDE-28 toxicity.

Treatments	Chla	Chlb	Total Chlorophyll	Soluble Sugars (mg g ⁻¹)
Control	0.95 ± 0.05i	0.52 ± 0.08h	1.48 ± 0.16i	5.48 ± 0.19h
Bd	1.45 ± 0.04c	0.80 ± 0.04b	2.26 ± 0.48bc	12.06 ± 0.24bc
Ca	1.31 ± 0.05e	0.72 ± 0.03d	2.04 ± 0.029e	9.92 ± 0.11d
Bd _{Ca}	1.73 ± 0.03a	0.95 ± 0.01a	2.69 ± 0.36a	14.69 ± 0.19a
Cd	0.77 ± 0.04kl	0.42 ± 0.064jk	1.20 ± 0.54kl	2.79 ± 0.32jk
DDT	0.87 ± 0.03k	0.48 ± 0.04j	1.36 ± 0.049k	3.18 ± 0.41j
Cd.DDT	1.50 ± 0.02l	1.28 ± 0.04k	1.78 ± 0.075l	3.41 ± 0.17l
Bd.Cd	1.27 ± 0.04e	0.70 ± 0.03de	1.98 ± 0.067e	9.37 ± 0.18de
Bd.DDT	1.38 ± 0.06ef	0.75 ± 0.01de	2.15 ± 0.052ef	9.75 ± 0.21de
Bd.Cd.DDT	1.20 ± 0.03h	0.66 ± 0.07fg	1.86 ± 0.047h	7.07 ± 0.17fg
Ca. Cd	1.13 ± 0.04g	0.62 ± 0.06fg	1.76 ± 0.036g	7.23 ± 0.17fg
Ca.DDT	1.24 ± 0.03gh	0.68 ± 0.04f	1.92 ± 0.075gh	7.62 ± 0.16f
Ca.Cd.DDT	1.05 ± 0.05ij	0.58 ± 0.06hi	1.64 ± 0.15ij	4.93 ± 0.18hi
Bd _{Ca} .Cd	1.55 ± 0.08b	0.85 ± 0.07bc	2.41 ± 0.094b	12.00 ± 0.14bc
Bd _{Ca} DDT	1.65 ± 0.04bc	0.90 ± 0.03b	2.58 ± 0.042bc	12.38 ± 0.24b
Bd _{Ca} .Cd.DDT	1.47 ± 0.07d	0.81 ± 0.04de	2.29 ± 0.075d	9.70 ± 0.19de

Values demonstrate means ± SD (n = 5). Different letters indicate significant difference among the treatments (P ≤ 0.05). C, control; Cd, Cadmium; Ca, Calcium.

increased. The enzyme activities were also improved by Ca treatment alone, but the level of upregulation was significantly lower than that of Bd. The amounts of all these enzymes shared a common pattern of elevation after exposure to Ca and Bd. Whereas significant reductions in the enzyme activities were observed after their exposure to the plant stressors, i.e., Cd and DDT. The mitigating function of Bd_{Ca}, however, was dominant, and it mitigated all the stress effects of Cd and DDT on *B. alboglabra* (Table 2).

Proline, Lycopene, β-Carotene, and Lutein Contents

Proline contents showed significant retardation in the plants treated with DDT (43.71%). However, a positive effect of Bd was recorded on the proline, resulting in a significant elevation of 46.18% in its contents as compared to the control treatment. The positive results of Bd on proline contents were also comparable with the positive results of Ca. Cd toxicity negatively affected lycopene, β-carotene, and lutein. Cd and DDT jointly abridged the proline contents more severely. However, all these biochemicals were substantially improved by the three treatments, namely, Bd, Ca, and Bd_{Ca} (Figure 4).

Water Potential, Leaf Osmotic Potential, and MSI

Exogenously applied Bd caused the leaf water potential of Kale plants to increase by 153.620%. Kale plants are grown in a toxic DDT environment, which, however, had an 8.69% lower potential for leaf water. Similarly, Cd application negatively impacted the leaf water potential and caused a significant reduction, whereas Ca and Bd_{Ca} treatments have demonstrated significant improvements in leaf water potential. The treatments also mitigated the Cd and DDT toxicity effectively. The leaf osmotic potential and MSI were also found following a similar pattern.

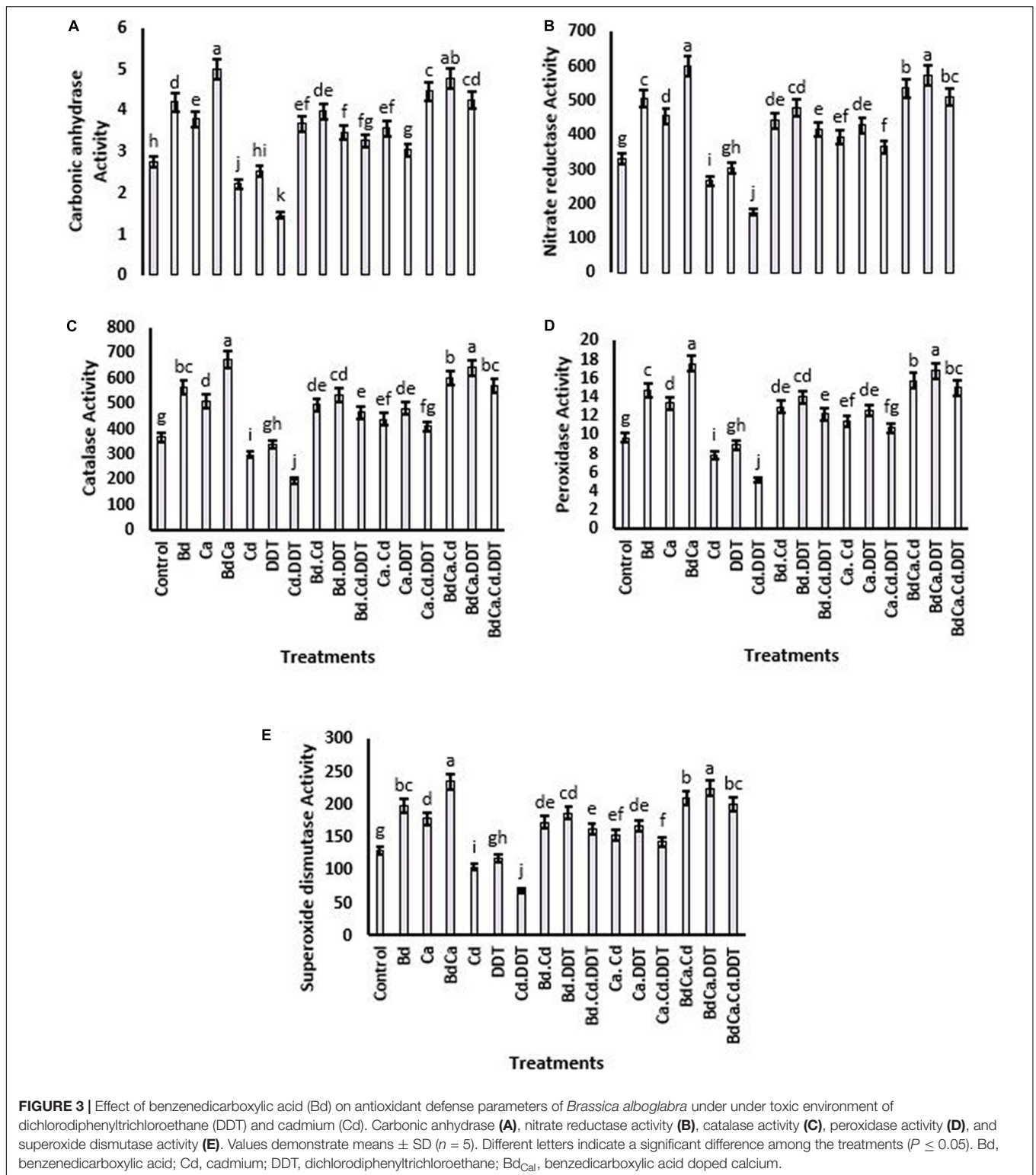
The stress effects of Cd and DDT toxicity were successfully mitigated by Bd_{Ca}, which was the best treatment used in the assays (Table 3).

Cd Contents

The Cd contents were recorded the highest in root tissues of the plants (22.21 μg/g), which were reduced to 20.41 μg/g in the shoots, 19.89 μg/g in the leaves, and ultimately 19.35 μg/g in the seeds when Kale plants were exposed to Cd. The TF value of Cd was 633.60, and MTI of Kale against Cd was 7.87, with a removal efficacy (RE) value of 13.09. DDT put the stress effects on Kale plants, significantly increasing the Cd contents in the plant tissues, elevated TF, decreased MTI, and reduced RE. The best performing treatment Bd_{Ca} showed reduced Cd content of 11.72 μg/g in the plant roots, 10.77 μg/g in shoots, 10.50 μg/g in leaves, and 10.21 μg/g in seeds under the influence of two stressors, namely, Cd and DDT. The treatment also showed a TF value of 334.40, an MTI value of 15.05, and an RE value of 71.61 (Table 3).

DDT Contents, TF, and Pollutant Tolerance Index

The DDT accumulation was higher in roots (8.39 μg/g) compared to shoots (5.59 μg/g), leaves (3.71 μg/g), and seeds (1.69 μg/g) under the DDT control treatment during the study. Meanwhile, the TF value was 182.04, along with the MTI value of 16.87 and the RE value of 173.28. Cd played the role of an enhancer for DDT accumulations (elevating DDT bioconcentration) in both plant tissues and seeds (2.44 μg/g). Both the Ca and Bd mitigated the stress caused by DDT and Cd and lowered down the DDT contents in plant tissues. The Cd application assisted the stress effects of DDT and caused a significant increase in its bioaccumulation in plant tissues. After the application of Bd, the DDT contents were recorded 5.33 μg/g



in roots, 3.55 $\mu\text{g/g}$ in shoots, 2.36 $\mu\text{g/g}$ in leaves, and 1.07 $\mu\text{g/g}$ in seeds of the Kale plants. It could reduce the TF value up to 115.62, whereas it improved the MTI value up to 26.59 and the RE value up to 584.82. The most promising treatment was BdCa

that did not allow DDT contents to exceed 5.39 $\mu\text{g/g}$ in the roots, 3.59 $\mu\text{g/g}$ in the shoots, 2.38 $\mu\text{g/g}$ in the leaves, and 1.08 $\mu\text{g/g}$ in the seeds of the Kale plants. It recorded a TF value of 116.85 with improved MTI and RE values (Table 4). The overall observation

TABLE 2 | Effect of benzenedicarboxylic acid (Bd) and calcium nanoparticles (Ca) on dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), glutathione-s-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPX), and ascorbate peroxidase (APX), in *Brassica albuglabra* under Cd stress, temperature stress, and BDE-28 toxicity.

Treatments	DHAR (nmol min ⁻¹ mg ⁻¹ protein)	MDHAR (nmol min ⁻¹ mg ⁻¹ protein)	GST (nmol g ⁻¹ FW)	GR (EU mg ⁻¹ protein)	GPX (μmol g ⁻¹ FW)	APX (EU mg ⁻¹ protein)
Control	103.00 ± 3.9h	54.20 ± 2.47h	37.10 ± 1.25h	3.98 ± 0.12h	53.14 ± 1.34f	2.21 ± 0.08f
Bd	157.59 ± 7.1c	82.93 ± 3.44bc	56.76 ± 1.27bc	6.09 ± 1.01d	81.30 ± 2.91c	3.38 ± 0.15bc
Ca	142.14 ± 5.61d	74.80 ± 2.1d	51.20 ± 1.87d	5.49 ± 0.13e	73.33 ± 1.75d	3.05 ± 0.05d
Bd _{Ca}	187.46 ± 2.8a	98.64 ± 1.11a	67.52 ± 1.9a	7.24 ± 0.03a	96.71 ± 0.99a	4.02 ± 0.02a
Cd	83.43 ± 3.67jk	43.90 ± 2.21jk	30.05 ± 0.81jk	3.22 ± 0.34j	43.04 ± 2.23g	1.79 ± 0.07g
DDT	94.76 ± 7.14j	49.86 ± 2.21j	34.13 ± 1.17j	3.66 ± 0.41hi	48.89 ± 1.43fg	2.03 ± 0.05fg
Cd.DDT	55.59 ± 3.1k	29.73 ± 1.14k	19.66 ± 0.99k	2.11 ± 0.05k	28.16 ± 0.64h	1.17 ± 0.08h
Bd.Cd	138.02 ± 8.62de	72.63 ± 2.32de	49.71 ± 1.14de	5.33 ± 0.28ef	71.21 ± 1.28de	2.96 ± 0.11de
Bd.DDT	149.35 ± 6.61de	78.59 ± 2.41de	53.80 ± 1.53de	5.77 ± 0.32de	77.05 ± 2.15cd	3.20 ± 0.08cd
Bd.Cd.DDT	129.78 ± 5.34fg	68.29 ± 2.14fg	46.75 ± 2.19fg	5.01 ± 0.31f	66.96 ± 3.17e	2.78 ± 0.06e
Ca. Cd	122.57 ± 4.74fg	64.50 ± 2.71fg	44.15 ± 1.22fg	4.74 ± 0.37fg	63.24 ± 1.98e	2.63 ± 0.09e
Ca.DDT	133.90 ± 3.67f	70.46 ± 1.34f	48.23 ± 1.77f	5.17 ± 0.61ef	69.08 ± 3.75de	2.87 ± 0.05de
Ca.Cd.DDT	114.33 ± 3.67hi	60.16 ± 3.11hi	41.18 ± 2.07hi	4.42 ± 1.01g	58.99 ± 1.63a	2.45 ± 0.09g
Bd _{Ca} .Cd	167.89 ± 3.67b	88.35 ± 2.49bc	60.47 ± 1.33b	6.49 ± 0.11c	86.62 ± 0.64b	3.60 ± 0.07b
Bd _{Ca} DDT	179.22 ± 3.67c	94.31 ± 2.42b	64.55 ± 2.16bc	6.93 ± 0.03ab	92.46 ± 1.38ab	3.85 ± 0.07ab
Bd _{Ca} .Cd.DDT	159.65 ± 3.67de	84.01 ± 2.21de	57.51 ± 2.84de	6.17 ± 0.34cd	82.40 ± 2.41bc	3.43 ± 0.06bc

Values demonstrate means ± SD (n = 5). Different letters indicate significant difference among the treatments (P ≤ 0.05). C, control; Cd, Cadmium; Ca, Calcium.

of the experiments was summarized in a schematic diagram to understand the positive effects of Bd_{Ca} and the mechanistic aspects behind them (Figure 5).

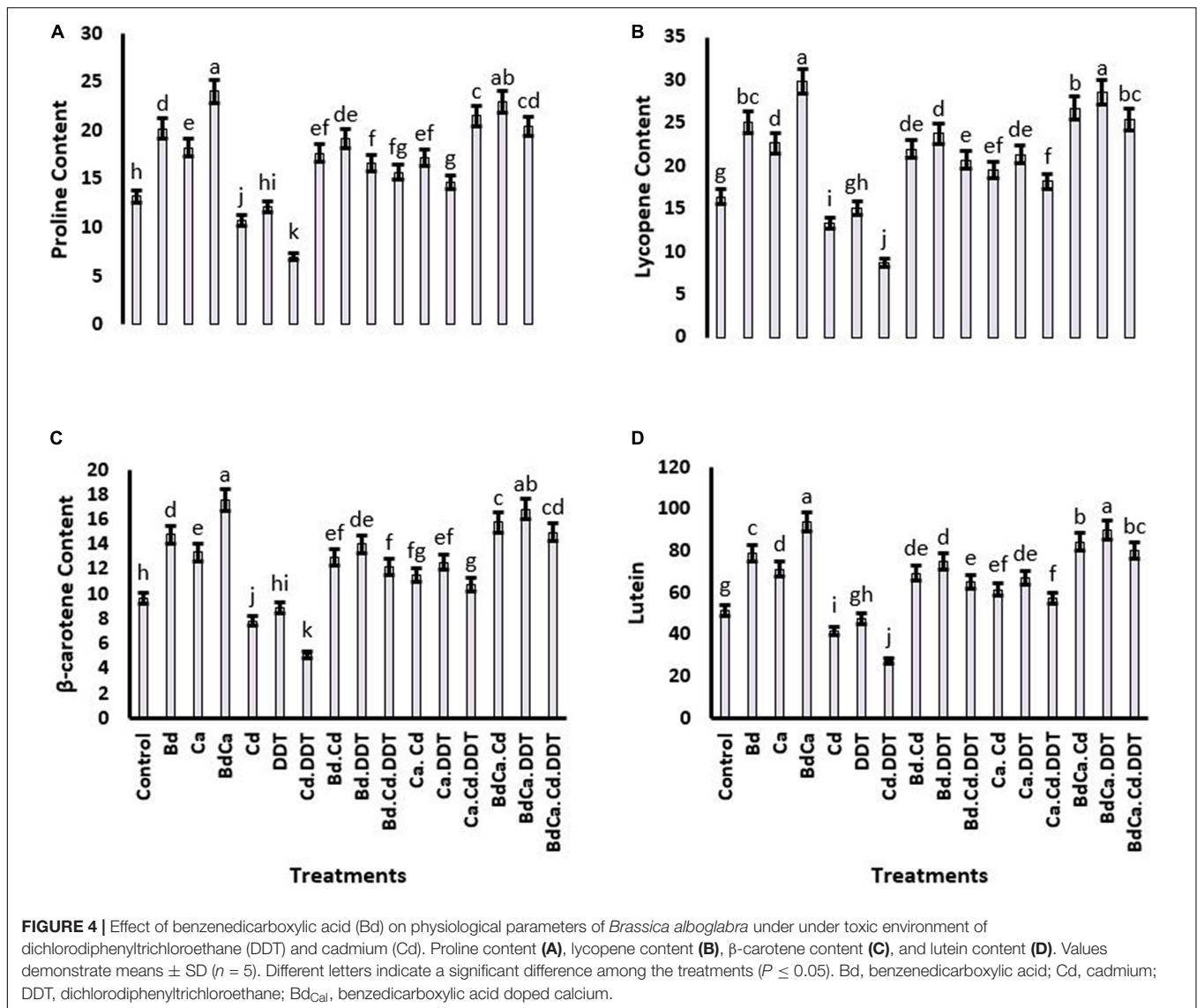
DISCUSSION

For the first time, the current investigation sets out a method for the sustainable exogenous delivery of Bd to a plant surface after Ca impregnation. Bd is a fungal metabolite primarily known for its role in the augmentation of cell defenses against pathogenic fungi (Ahmad et al., 2014a,b, 2019). The reaction of the plant to Bd is purely dose- and duration-dependent. The early studies reported that this compound improved the nutritional profiles of tomato fruits. However, with the high water solubility, it cannot be applied to plant surfaces in rainy and humid environments. To ensure the sustained availability of Bd at plant surfaces, a formulation development is necessary. This study remains an outstanding approach in the field of plant protection and cell physiology. In addition, Bd has a tendency to change the physiological processes and genetic controls of plants (Khan et al., 2019; Ahmad et al., 2020a).

Stress factors (e.g., DDT) induce metabolic imbalances and encourage EL and oxidative stress, contributing to the accumulation of solutes in the form of adaptive cellular responses (Jan et al., 2012; Shafique et al., 2014). This lays the basis for the increased coefficient of bioconcentration of DDT in various *Brassica* plant tissues. In addition, the analysis first defines the TF of DDT in Kale plants, elaborating the fate of the pollutant absorbed into the edible plant (Rignell-Hydbom et al., 2007; Khan et al., 2017). Bd is involved in initializing the expression of the genetic cascade associated with starch and sugar, which essentially determines the energy economy of the plant.

This property of Bd has been used during stress conditions to harmonize the physiological balances. In addition, the scientific research investigated the mitigation actions of Ca and Bd through the reharmonization of stress markers, such as EL, LRWC, gaseous exchange, antioxidant defenses, and the activities of enzymes. The primary tools of environmental toxicants to slow plant growth and metabolism are EL, *Ci*, reduced *Pn*, and leaf water potential. Cd toxicity also exerts similar stress symptoms on the physiology of plants (Faiz et al., 2021). The study recommends Bd_{Ca}, which has the potential to rehabilitate cellular homeostasis, as an effective plant metabolism modulator. It was evident that Bd_{Ca} increased the photosynthetic pigments (Chl *a* and Chl *b*), *Pn*, and total soluble sugars, which demonstrated the full-swing rehabilitation of Kale photosynthetic function, which was reduced by Cd and DDT. During the current investigation, increased chlorophyll biosynthesis subsequently increased the photosynthetic activity and augmented development in Bd_{Ca}-applied plants.

The least objective of a plant is to ensure its survival through the devastating period of stress, and the least objective of a farmer is to get enough yield to cover the cultivation costs (Yasin et al., 2018b). Both of these objectives can be achieved by improving plant tolerance against stressors (Zaheer et al., 2016). Therefore, this study considered the tolerance index against both the pollutant DDT and the metal Cd. Both the stress mitigators (i.e., Ca, Bd) and their combined form (Bd_{Ca}) successfully achieved phoenix tolerance indices DDT and Cd. Removing the pollutants and heavy metals is the safest method for remediating environmental toxins and safeguarding the food chain for consumers, including humans (Shah et al., 2020). It also makes the toxins and pollutants to be exposed to environmental deteriorations for natural degradation (Liu et al., 2013; Abbas et al., 2020). This study introduced a unique technique based on



Bd-impregnated Ca, which successfully hampered the entry of DDT into the food chain, which would certainly be detoxified overtime under natural degradation.

In addition, the single and combined effect of Ca and Bd resulted in beneficial physiochemical changes in plant cells. A few studies have previously used Ca for topical delivery of RNAi to make plants immune to pathogens (Mitter et al., 2017). Even, there was no ultimate conclusion about how the application of nanoparticles will respond to plant growth. This study highlighted the supporting role of Ca nanoparticles in plant growth for the first time. All the tested growth parameters of the Kale plants were in favor of the technique. It also elevated the enzyme activities responsible for antioxidant defenses, metabolic processes, and other physiological responses without a negative effect on plant growth. The study showed that Ca had an enormous potential to be used under stress conditions as a plant growth enhancer. A beneficial

aspect of Ca explored in this study is getting no adverse cross talk with Bd.

The importance of LRWC serves as a biomarker of plant-water relationships (Wang et al., 2019; Ahmad et al., 2021a). There are detrimental effects of Cd stress on both osmotic ability and water (Khan et al., 2018; Hafeez et al., 2020). The LRWC values of *Brassica* plants were also negatively impacted by Cd, suggesting decreased drought tolerance. All the three treatments, namely, Ca, Bd, and BdCa, greatly improved LRWC during the current investigation, proving a good option for high precipitation areas and areas adversely affected by Cd contamination. Lipid peroxidation-induced membranous injuries deteriorate cell metabolic processes and serve as a biomarker of cellular injuries in stressed plants. Similarly, the increased amount of ROS triggers the necrosis of cells in stressed plants (Arshad et al., 2016; Tariq et al., 2021). However, under stress, Ca and Bd-applied plants showed a decreased synthesis

TABLE 3 | Effect of benzenedicarboxylic acid (Bd) and calcium nanoparticles (Ca) on water potential, leaf osmotic potential, membrane stability index (MSI), root and shoot Cd uptake, translocation factor (TF) of Cd and metal tolerance index (MTI) of Cd in *Brassica alboglabra* under Cd stress, temperature stress, and BDE-28 toxicity.

Treatments	Water potential	Leaf osmotic potential	MSI	Root Cd ($\mu\text{g g}^{-1}$)	Shoot Cd ($\mu\text{g g}^{-1}$)	Leaf Cd ($\mu\text{g g}^{-1}$)	Seed Cd ($\mu\text{g g}^{-1}$)	TF _{Cd}	MTI	RE _{Cd} (%)
Control	0.69 ± 0.04h	0.860 ± 0.04h	0.93 ± 0.03f	-	-	-	-	-	-	-
Bd	1.06 ± 0.19d	1.316 ± 0.03d	1.42 ± 0.04c	-	-	-	-	-	-	-
Ca	0.95 ± 0.03e	1.187 ± 0.02e	1.28 ± 0.01d	-	-	-	-	-	-	-
Bd _{Ca}	1.26 ± 0.1a	1.565 ± 0.04a	1.69 ± 0.01a	-	-	-	-	-	-	-
Cd	0.56 ± 0.03j	0.697 ± 0.01j	0.75 ± 0.01g	22.21 ± 0.15b	20.41 ± 0.03b	19.89 ± 0.15b	19.35 ± 0.03b	633.60 ± 5.01b	7.87 ± 3.5b	13.09 ± 0.08b
DDT	0.63 ± 0.04hi	0.791 ± 0.07hi	0.86 ± 0.04fg	-	-	-	-	-	-	-
Cd,DDT	0.37 ± 0.03k	0.456 ± 0.03k	0.49 ± 0.02h	26.41 ± 0.21a	24.27 ± 0.2a	23.65 ± 0.21a	23.01 ± 0.2a	753.28 ± 8.04a	5.15 ± 3.1a	8.085 ± 0.51a
Bd,Cd	0.92 ± 0.03ef	1.152 ± 0.04ef	1.25 ± 0.03de	11.97 ± 0.1cd	11.00 ± 0.1cd	10.72 ± 0.1cd	10.43 ± 0.1cd	341.44 ± 0.01cd	13.01 ± 6.9cd	67.76 ± 1.24cd
Bd,DDT	1.00 ± 0.03de	1.247 ± 0.03de	1.35 ± 0.02cd	-	-	-	-	-	-	-
Bd,Cd,DDT	0.87 ± 0.04f	1.084 ± 0.03f	1.17 ± 0.01e	10.24 ± 0.48de	9.41 ± 0.01d	9.17 ± 0.48de	8.92 ± 0.01de	292.16 ± 0.03d	12.23 ± 5.8de	49.28 ± 1.33d
Ca, Cd	0.82 ± 0.02fg	1.023 ± 0.01fg	1.11 ± 0.02e	10.98 ± 0.38d	10.09 ± 0.02cd	9.83 ± 0.38d	9.57 ± 0.02d	313.28 ± 0.01cd	11.55 ± 3.7d	55.055 ± 2.02cd
Ca,DDT	0.90 ± 0.04ef	1.118 ± 0.02ef	1.21 ± 0.01de	-	-	-	-	-	-	-
Ca,Cd,DDT	0.77 ± 0.04g	0.955 ± 0.02g	1.03 ± 0.01a	8.51 ± 0.19f	7.82 ± 0.01e	7.62 ± 0.19f	7.42 ± 0.21f	242.88 ± 0.01e	10.78 ± 6.1f	36.575 ± 1.14e
Bd _{Ca} ,Cd	1.12 ± 0.03c	1.402 ± 0.02c	1.52 ± 0.01b	12.22 ± 0.38c	11.23 ± 0.21c	10.94 ± 0.38c	10.64 ± 0.03c	348.48 ± 0.03c	15.83 ± 2.96c	90.09 ± 2.35c
Bd _{Ca} , DDT	1.20 ± 0.03ab	1.496 ± 0.01ab	1.62 ± 0.02ab	-	-	-	-	-	-	-
Bd _{Ca} ,Cd,DDT	1.07 ± 0.03cd	1.333 ± 0.03cd	1.44 ± 0.02bc	11.72 ± 0.4cd	10.77 ± 0.03cd	10.50 ± 0.4cd	10.21 ± 0.01cd	334.40 ± 0.03cd	15.05 ± 6.3cd	71.61 ± 3.14cd

Values demonstrate means ± SD (n = 5). Different letters indicate significant difference among the treatments ($P \leq 0.05$). C, control; Cd, Cadmium; Ca, Calcium.

of ROS (H_2O_2). Furthermore, the amount of MDA, which is the final by-product of lipid peroxidation, was decreased by Bd_{Ca} supplementation. Similarly, oxidative damage to cells and lipid membranes is triggered by a higher H_2O_2 level in stressed plants (Farooq et al., 2013; Sattar et al., 2020). The EL caused by the weakened cell membrane decreases the development of plant growth and biomass. Several studies have shown that the upregulated antioxidant machinery activity decreases the synthesis of H_2O_2 in stressed plants (Farooq et al., 2013; Sattar et al., 2020; Ahmad et al., 2021b). Superoxide is detoxified by the action of SOD into a less injurious H_2O_2 , which is eventually transformed by the activity of POD, catalase (CAT), and APX into H_2O . By improving the function of the antioxidative system in stressed plants, the exogenous application of Bd_{Ca} improved stress tolerance. In addition to the decreased level of EL, H_2O_2 , and MDA, the upregulation in antioxidant machinery has highlighted the favorable role of Bd_{Ca} in reducing oxidative injuries and controlling redox homeostasis in stressed plants.

The SOD is known as one of the key antioxidant defenses of plant cells, which is essential for survival under the conditions of stress (Askary et al., 2017). While SOD and CAT are primarily localized in the plant cell peroxisomes and mitochondria, they have shown a significant response to stress conditions (Shereefa and Kumaraswamy, 2016). The stress responses in Kale plants, however, were mitigated by Bd_{Ca} treatment. A comprehensive interrelation between the enzyme activities of Bd, Ca, Bd_{Ca}, ROS, MDA, and antioxidants is given in the current investigation. It can be inferred that all three treatments (i.e., Bd, Ca, and Bd_{Ca}) have improved plant defenses against Cd stress and DDT toxicity by considering the overall physiological responses of the plant. However, in this respect, the most promising formulation was Bd_{Ca}. The scavenging of H_2O_2 was a primary feature of Kale plants thriving under stress conditions in the plant cells. The key source of ROS is H_2O_2 , and one of the most important substrates for scavenging H_2O_2 is ascorbate (Ibrahim et al., 2016; Nahar et al., 2017). The greater biosynthesis of ROS than that which is scavenged (by the antioxidant system) contributes to irreversible oxidative damages to the plant cells. The non-enzymatic antioxidant protection mechanism contains ascorbate, glutathione, and carotenoids. However, the main components of the enzymatic antioxidant mechanism are GST, GR, GPX, DHAR, MDHAR, APX, SOD, and CAT. A dual role to improve the oxidative defense mechanism of Kale plants has been performed by Bd_{Ca}. It raised the activity of antioxidants to further increase the protective layers of plants against oxidative damages.

Researchers have long been involved in increasing the proline and carotenoid content of edible plants (i.e., β -carotene, lycopene, and lutein). The pigmented elements of plants that contribute to the photosynthetic machinery and perform a defensive role against photodamage are carotenoids (Tan and Norhaizan, 2019). Foods rich in carotenoids defend humans against age-related illnesses. Lycopene is a major carotenoid of Kale plants with a distinctive red color and unique antioxidant properties. Proline is a primary component of the machinery for protein biosynthesis that determines the cell structure, metabolism, profile of nutrition, wound healing, antioxidant reactions, and responses to protection (Wu et al., 2011;

TABLE 4 | Effect of benzenedicarboxylic acid (Bd) and calcium nanoparticles (Ca) on water potential, leaf osmotic potential, membrane stability index (MSI), root and shoot Cd uptake, translocation factor (TF) of Cd and pollutant tolerance index (PTI) of Cd in *Brassica alboglabra* under Cd stress, temperature stress, and BDE-28 toxicity.

Treatments	Root DDT ($\mu\text{g g}^{-1}$)	Shoot DDT ($\mu\text{g g}^{-1}$)	Leaf DDT ($\mu\text{g g}^{-1}$)	Seed DDT ($\mu\text{g g}^{-1}$)	TF _{DDT}	PTI	RE _{DDT} (%)
Control	–	–	–	–	–	–	–
Bd	–	–	–	–	–	–	–
Ca	–	–	–	–	–	–	–
Bd _{Ca}	–	–	–	–	–	–	–
Cd	–	–	–	–	–	–	–
DDT	8.39 ± 0.19b	5.59 ± 0.1b	3.71 ± 0.15b	1.69 ± 0.02b	182.04 ± 4.47b	16.87 ± 6.9g	173.28 ± 3.56g
Cd.DDT	12.13 ± 0.21a	8.09 ± 0.2a	5.37 ± 0.21a	2.44 ± 0.2a	263.22 ± 7.63a	9.72 ± 3.1h	75.81 ± 4.37h
Bd.Cd	–	–	–	–	–	–	–
Bd.DDT	5.33 ± 0.43cd	3.55 ± 0.01cd	2.36 ± 0.4cd	1.07 ± 0.01cd	115.62 ± 3.17cd	26.59 ± 6.3c	584.82 ± 4.74c
Bd.Cd.DDT	4.71 ± 0.48d	3.14 ± 0.01d	2.08 ± 0.48cd	0.95 ± 0.01cd	102.09 ± 4.03d	23.11 ± 5.8de	462.08 ± 5.31de
Ca. Cd	–	–	–	–	–	–	–
Ca.DDT	4.42 ± 0.38de	2.95 ± 0.03de	1.96 ± 0.38d	0.89 ± 0.03d	95.94 ± 2.13de	23.84 ± 6.1d	465.69 ± 9.11d
Ca.Cd.DDT	3.91 ± 0.1e	2.61 ± 0.21e	1.73 ± 0.38de	0.79 ± 0.21de	84.87 ± 1.17e	20.36 ± 3.5f	342.95 ± 8.06f
Bd _{Ca} .Cd	–	–	–	–	–	–	–
Bd _{Ca} DDT	5.44 ± 0.15c	3.63 ± 0.03c	2.41 ± 0.1c	1.09 ± 0.03c	118.08 ± 1.98c	31.91 ± 2.96a	794.2 ± 5.73a
Bd _{Ca} .Cd.DDT	5.39 ± 0.38cd	3.59 ± 0.02cd	2.38 ± 0.19cd	1.08 ± 0.1cd	116.85 ± 2.44cd	28.43 ± 3.7b	671.46 ± 4.24b

Values demonstrate means ± SD (n = 5). Different letters indicate significant difference among the treatments (P ≤ 0.05). C, control; Cd, Cadmium; Ca, Calcium.

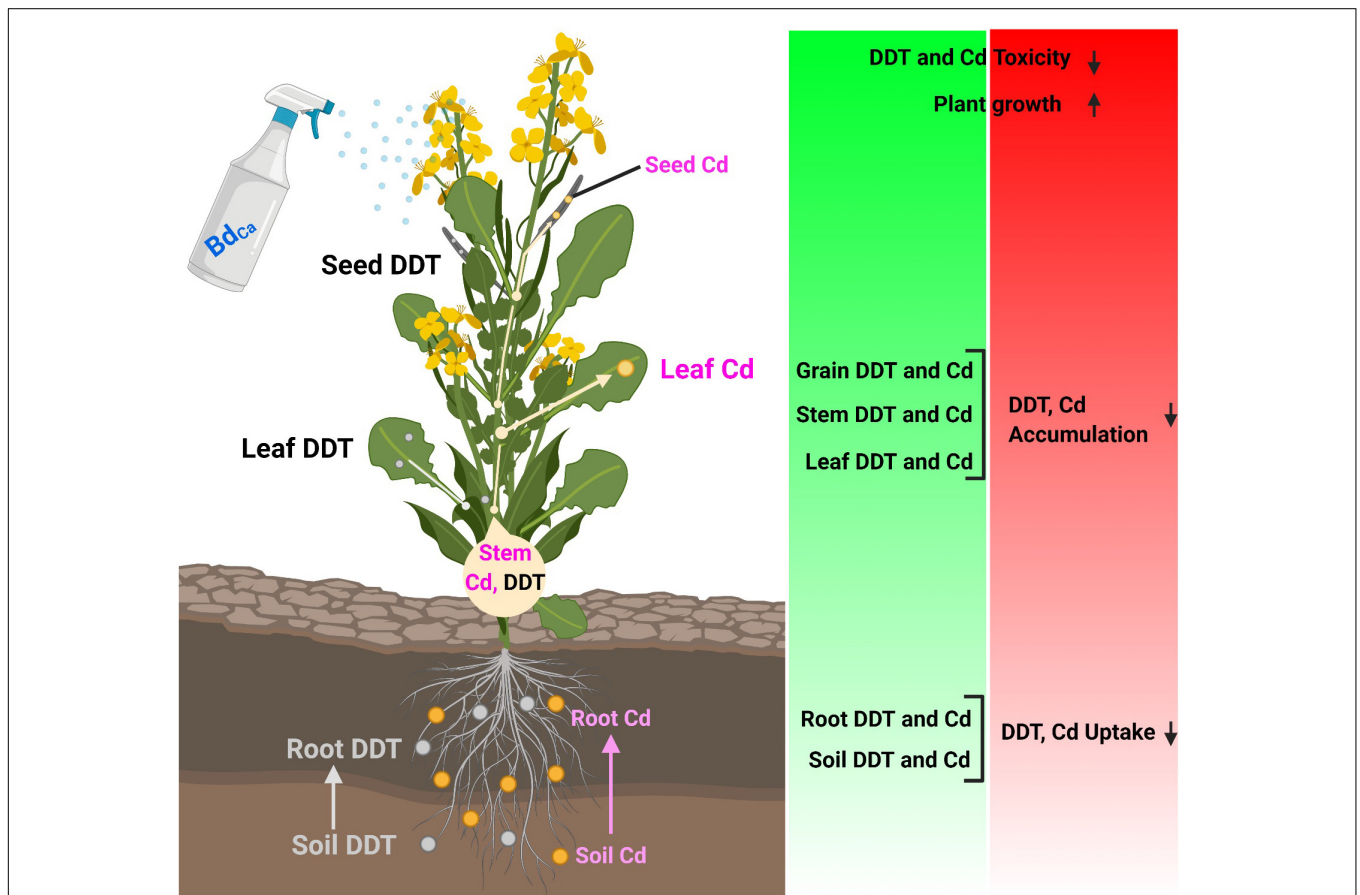


FIGURE 5 | Illustration of mechanistic role of calcium nanoparticles impregnated with benzenedicarboxylic acid in alleviation of combined stress of DDT and Cd in *Brassica alboglabra* by modulating bioaccumulation, antioxidative machinery and osmoregulators.

Shaddad et al., 2013). Proline has a pivotal role in the stability of the arrangement of macromolecules and the turgidity of the structure of the cell membrane in stressed plants. Proline scavenges ROS in plants and attenuates tension. Bd_{Ca} successfully increased the proline and carotenoid contents in Kale plants under various stress conditions during this study. It hardened the plants and made them tolerate DDT toxicity and Cd stress. The increased concentration of DDT in plant roots growing in a toxic DDT environment is because vacuole and root cell wall can accumulate and retain higher POP contents compared to the shoot cells. Due to modified homeostasis, the exogenous application of Bd_{Ca} decreased the DDT content in plants. Furthermore, all the advantages of reducing the content of DDT in Kale leaves and Kale seeds are the achievements of the study. Reducing the toxins and contaminants in the seeds of the Kale plants will be of much assistance to prevent humans from DDT. Given the essence of transfer, persistence, bioaccumulation, and lethal effects of DDT, the treatment of Bd_{Ca} achieved a hallmark of preventing the entry into the human food chain. In addition, the application of Bd_{Ca} decreased the bioaccumulation of the Kale roots, hampering the transfer of DDT from soil to food at the first level. The analysis can be established to look for the bioconcentration activity of POPs in other food plants.

This study discloses a novel strategy to grow healthier food crops in a contaminated environment. The large-scale production and application of Bd_{Ca} can mitigate chemical stress and heavy metal stress, and *Brassica* produces more crop yields with the least accumulation of POPs. It can be a very beneficial technique of crop cultivation in the contaminated soil and air. However, some detailed studies are required to verify the effects of Bd_{Ca} on some other crop plants.

CONCLUSION

It can be concluded that the growth and physiological attributes of Kale were successfully improved by Ca- and Bd-dependent Bd_{Ca} under various stress conditions caused by Cd and DDT

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pollution. By reducing the biosynthesis of ROS, enhancing the antioxidative mechanism, and modulating the synthesis of osmoregulators, the exogenously applied Bd_{Ca} increased plant resistance to the combined abiotic stressors. In addition, Bd_{Ca} inhibited the absorption of DDT in plant tissues and decreased its TF. Therefore, the novel treatment of Bd_{Ca} is recommended for relieving multiple stresses and for a sustainable crop production system. However, further field experiments are compulsory to evaluate the efficacy of Bd_{Ca} in Kale plants and other crop 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

SM and IS: perform experiments. AA and WA: design research. IS: perform WS statistical analysis. NY: writing. AS: review and drafting. MS and SA: writing and funding acquisition. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

The authors would like to extend their sincere appreciation to the Researchers Supporting Project Number (RSP-2021/194), King Saud University, Riyadh, Saudi Arabia.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2022.825829/full#supplementary-material>

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