

MITIGATING CADMIUM-INDUCED TOXICITY IN TURNIP (*BRASSICA RAPA*) THROUGH EXOGENOUS APPLICATION OF VANILLIC ACIDNadia Jabeen^{*1}, Muhammad Abbas Khan², Bushra Safdar³, Ameer Jan⁴^{*1}Department of Agriculture, Hazara University Mansehra²Department of Horticulture, Balochistan Agriculture College Quetta³Department of Agronomy, University of Agriculture Faisalabad⁴University of Makran¹nadia_khan909090@yahoo.com, ²muhammadabbaskhan1121@gmail.com,³bushrasafdar1999@gmail.com, ⁴ameerjan@uomp.edu.pk¹Orcid ID: 0000-0001-6617-8301DOI: <https://doi.org/10.5281/zenodo.19678022>**Keywords**

cadmium toxicity, vanillic acid, *Brassica rapa*, oxidative stress, glyoxalase system, antioxidant defense, phytochelatins, heavy metal tolerance, phenolic acids, phytoremediation

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Abstract

Cadmium (Cd) contamination in agricultural soils poses a severe threat to crop productivity and food safety, particularly in root vegetables like turnip (*Brassica rapa*), where it induces morphological stunting, biomass reduction, photosynthetic impairment through chlorophyll degradation, oxidative stress via reactive oxygen species (ROS) overproduction, lipid peroxidation (elevated MDA), and methylglyoxal (MG) accumulation leading to cytotoxicity. This toxicity disrupts nutrient homeostasis, water relations, and enzyme activities while overwhelming endogenous antioxidant and glyoxalase defense systems. Exogenous application of vanillic acid (VA), a phenolic compound derived from the phenylpropanoid pathway, emerges as an effective phytoprotectant strategy. VA pretreatment (typically 2–4 mM) mitigates Cd-induced damage by restoring growth parameters and biomass, preserving chlorophyll content and photosynthetic efficiency, improving stomatal conductance and leaf turgidity, and reducing physiological drought symptoms. Biochemically, VA upregulates key antioxidant enzymes (SOD, CAT, POD, APX) and the ascorbate-glutathione (AsA-GSH) cycle, lowers H₂O₂ and MDA levels, enhances glyoxalase I and II activities for efficient MG detoxification, and promotes accumulation of osmoprotectants like proline. Furthermore, VA strengthens metal sequestration by boosting phytochelatin synthesis and vacuolar compartmentalization, modulates heavy metal transporter genes (e.g., ZIP, NRAMP, HMA families), and integrates with hormone signaling (ABA, SA, NO) and secondary metabolism for cell wall reinforcement. Comparative analyses highlight VA's advantages over other agents like biochar, selenium, or citric acid due to its dual role in internal tolerance and minimal environmental footprint. These mechanisms collectively enhance Cd tolerance, support safer food production, and improve phytoremediation potential in *Brassica rapa*. Future directions include nano-formulations for targeted delivery, genetic engineering of the VA biosynthetic pathway, and exploration of rhizosphere microbiota synergies. Overall, exogenous vanillic acid offers a sustainable, eco-

friendly approach to counteract cadmium toxicity, ensuring agricultural resilience and food security in contaminated regions.

1. INTRODUCTION

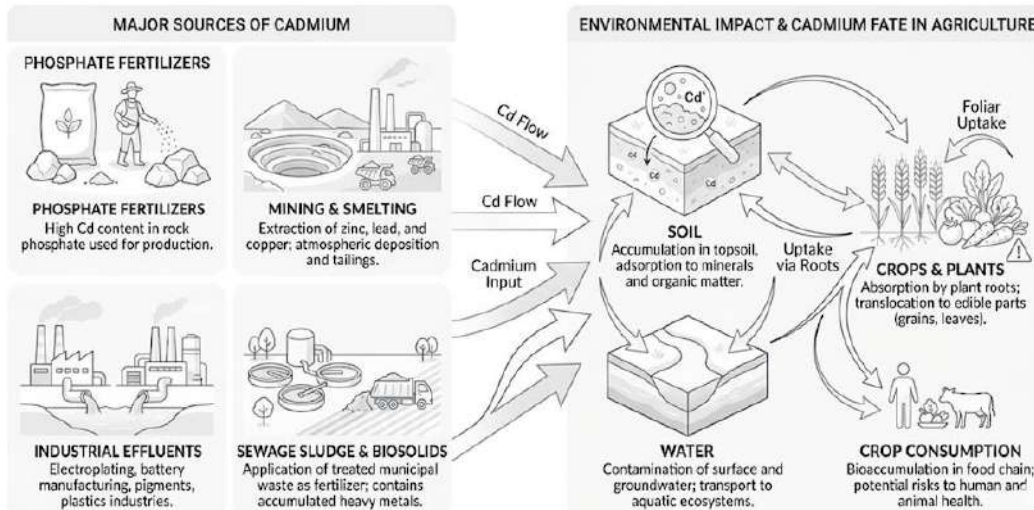
The contemporary agricultural landscape is increasingly defined by the dual challenge of meeting the nutritional demands of a burgeoning global population while contending with the pervasive threat of heavy metal pollution (Kamal et al., 2023). Among the myriads of environmental contaminants, cadmium (Cd) stands out as a non-essential, highly mobile, and persistent toxicant that poses significant risks to both plant health and human well-being (Vitelli et al., 2024). Within the Brassicaceae family, *Brassica rapa* (turnip) serves as a critical food source and a potential candidate for phytoremediation due to its high biomass and inherent ability to accumulate heavy metals (Boysan Canal et al., 2023). However, the accumulation of cadmium in turnip tissues leads to severe physiological, biochemical, and morphological disturbances, ultimately impairing yield and quality (Raza et al., 2020). Recent research has pivoted toward the use of exogenous phytoprotectants, specifically phenolic acids like vanillic acid (VA), to alleviate cadmium-induced stress (Bhuyan et al., 2020). Vanillic acid, a derivative of benzoic acid, exhibits potent antioxidant and signaling properties that modulate plant defense systems, including the

antioxidant machinery and the glyoxalase pathway, to combat the deleterious effects of metal toxicity (Parvin et al., 2024).

2. Global Environmental Impact and Cadmium Dynamics in Soil-Plant Systems

Cadmium pollution is a byproduct of both natural geological processes and intensive anthropogenic activities. The primary drivers of soil cadmium enrichment include the discharge of industrial effluents, mining and smelting operations, atmospheric deposition from fossil fuel combustion, and the widespread application of cadmium-containing phosphate fertilizers and sewage sludge in agricultural practice (Kubier et al., 2019). Unlike organic pollutants, cadmium is non-biodegradable and possesses a biological half-life in soil that can span several decades, typically ranging from 10 to 30 years (Genchi et al., 2020). Its high solubility and bioavailability in soil matrices facilitate its rapid uptake by plant roots, making it a primary entry point into the human food chain (Haider et al., 2021). Cadmium contamination in agricultural soils originates from multiple anthropogenic activities (Figure 1). Understanding these sources is essential to contextualize the cadmium-induced stress observed in *Brassica rapa*.

Figure 1: Sources and Environmental Impact of Cadmium in Agricultural Systems



The mobility of cadmium in the soil is influenced by several factors, including soil pH, organic matter content, and the presence of competing cations. For instance, an increase in soil pH through the application of amendments like biochar can reduce the bioavailability of Cd^{2+} ions by promoting their adsorption onto soil colloids or the formation of insoluble precipitates (Yuan et al., 2019). Conversely, in acidic soils,

cadmium remains highly mobile and is readily transported via the xylem to the aerial parts of the plant. This high soil-to-plant transfer ratio is particularly concerning for leafy and root vegetables like *Brassica rapa*, which can accumulate significant concentrations of cadmium in their edible tissues without immediately exhibiting lethal symptoms (Shahid et al., 2017).

Table 1. Sources of Cadmium Pollution and Their Environmental Impact

Source of Cadmium Pollution	Mechanism of Soil Contamination	Impact on Agricultural Sustainability
Phosphate Fertilizers	Gradual accumulation through repeated application (Huang et al., 2017)	Long-term degradation of soil quality and fertility (Noor et al., 2024)
Mining & Smelting	High-concentration localized deposition of metal dust (Noor et al., 2024)	Transformation of arable land into toxic zones (Huang et al., 2017)
Sewage Sludge Irrigation	Introduction of soluble metal complexes into the rhizosphere (Noor et al., 2024)	Increased bioavailability and uptake by food crops (Bisht & Garg, 2022)
Industrial Effluents	Direct discharge of liquid waste containing metal ions (Noor et al., 2024)	Contamination of groundwater and surface water irrigation (Kumar et al., 2024)

3. Physiological and Morphological Disruptions in Brassica rapa Under Cadmium Stress

The exposure of Brassica rapa to toxic levels of cadmium results in a cascade of morphological and physiological impairments that vary according to the dosage, duration of exposure,

and the developmental stage of the plant (Huang et al., 2017). Early indicators of cadmium toxicity include the inhibition of seed germination and the retardation of seedling growth. Morphological abnormalities such as root browning, a reduction in the number of lateral

roots, and the death of root tips are commonly observed, reflecting the direct impact of cadmium on the root meristematic activity (Bisht & Garg, 2022).

3.1 Impact on Biomass and Growth Parameters

In Brassica species, cadmium stress leads to a significant reduction in fresh and dry biomass, shoot height, and root length. The reduction in biomass is a direct consequence of impaired nutrient uptake and the diversion of metabolic energy toward defense and detoxification processes (D'Alessandro et al., 2013). Cadmium interferes with the absorption and distribution of essential minerals like calcium (Ca²⁺), potassium (K⁺), magnesium (Mg²⁺), iron (Fe²⁺), and zinc (Zn²⁺) due to its chemical similarity to these divalent cations (Mahmud et al., 2020).

3.2 Photosynthetic Impairment and Chloroplast Degradation

One of the most severe physiological consequences of cadmium toxicity in *Brassica rapa* is the deregulation of the photosynthetic apparatus. Cadmium induces the premature senescence of chloroplasts and destabilizes the pigment-protein complexes (Hasan et al., 2017). It promotes the degradation of chlorophyll a, chlorophyll b, and carotenoids, often by substituting the central magnesium (Mg²⁺) ion in the porphyrin ring of the chlorophyll molecule, forming ineffective Cd-Chl complexes (Küpper et al., 2007). This process is accompanied by stomatal closure, which restricts CO₂ assimilation and reduces the efficiency of the photosynthetic electron transport chain (Rucińska-Sobkowiak, 2016).

Metabolomic studies in *Brassica* suggest that cadmium-stressed plants may rely on an increased photorespiration rate as a compensatory mechanism to prevent the over-reduction of the electron transport chain and subsequent photoinhibition (Zhang et al., 2023). However, prolonged photorespiration leads to the depletion of CO₂ and the accumulation of oxygen, which further fuels the production of reactive oxygen species (ROS) and exacerbates

oxidative stress within the leaf tissues (Voss et al., 2013).

3.3 Water Relations and Transpiration

Cadmium stress significantly disrupts the water status of *Brassica rapa*, leading to reduced transpiration rates and a decline in leaf relative turgidity. The inhibition of water uptake by the roots, combined with stomatal closure, creates a state of physiological drought (Rahman et al., 2024). In some Brassica species, exposure to abiotic stress leads to anatomical changes, such as a reduction in the diameter of xylem vessels and an increase in epidermal thickening, as an adaptation to minimize water loss (Parmar et al., 2020). However, these changes also limit the upward transport of nutrients, further compromising the plant's metabolic health (Mishra et al., 2023).

4. Biochemical Mechanisms of Cadmium Toxicity: Oxidative Stress and Glycation

The cellular toxicity of cadmium in *Brassica rapa* is largely mediated through the indirect generation of oxidative stress and the accumulation of cytotoxic metabolic byproducts (Mishra et al., 2023). While cadmium is not a redox-active metal and cannot engage in Fenton-like reactions, it triggers a "domino effect" of molecular mechanisms that lead to a massive overproduction of reactive oxygen species (ROS) (Zhao et al., 2023).

4.1 Reactive Oxygen Species (ROS) and Lipid Peroxidation

Cadmium stress disrupts the function of electron transport chains in mitochondria and chloroplasts, leading to the leakage of electrons and the formation of superoxide radicals (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radicals (OH[•]). The accumulation of these ROS molecules initiates chain reactions that damage lipids, proteins, and nucleic acids (Unsal et al., 2020). Lipid peroxidation, measured by the accumulation of malondialdehyde (MDA), is a hallmark of cadmium toxicity, reflecting the loss

of cellular membrane integrity, fluidity, and permeability (Hu et al., 2023).

4.2 Inactivation of Enzymes and Affinity for Thiols

A primary mechanism of cadmium toxicity is its high affinity for sulfur-containing functional groups, particularly thiol (-SH) groups. Cadmium binds to these groups in essential enzymes and structural proteins, leading to their inactivation or denaturation (Noor et al., 2024). This is particularly damaging to the antioxidant defense system, as cadmium can inhibit the activities of ROS-detoxifying enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) (Sindhushree et al., 2025). The concurrent increase in ROS production and the inhibition of detoxifying enzymes create a state of severe oxidative imbalance.

4.3 Methylglyoxal (MG) Toxicity and the Glyoxalase System

Cadmium stress also leads to the elevated production of methylglyoxal (MG), a highly reactive dicarbonyl compound formed as a byproduct of glycolysis and other metabolic pathways (Hasanuzzaman et al., 2017). MG is a potent cytotoxic agent that can glycate proteins and DNA, leading to the formation of advanced glycation end-products (AGEs) and inducing oxidative damage (Yan et al., 2023). To mitigate MG toxicity, plants utilize the glyoxalase system, which consists of two enzymes: glyoxalase I (Gly I) and glyoxalase II (Gly II) (Yan et al., 2018). These enzymes work in tandem, using reduced glutathione (GSH) as a cofactor, to convert MG into non-toxic D-lactate (Yan et al., 2023). However, under severe cadmium stress, the endogenous glyoxalase system in *Brassica rapa* may be insufficient to prevent MG accumulation, leading to cellular injury and growth arrest (Jung et al., 2021).

5. Vanillic Acid: Chemistry, Biosynthesis, and Role as a Phytoprotectant

Vanillic acid (VA), or 4-hydroxy-3-methoxybenzoic acid, is a phenolic acid that serves as a vital secondary metabolite in many plant species. It is an oxidized derivative of vanillin and is characterized by its high degree of hydroxylation, which contributes to its robust radical-scavenging and antioxidant capabilities (Beyer, 2025).

5.1 Biosynthetic Origin and Phenylpropanoid Pathway

In plants, vanillic acid is synthesized through the shikimic acid and phenylpropanoid pathways. These pathways are frequently upregulated in response to abiotic stressors, including heavy metal toxicity, drought, and salinity, leading to the accumulation of various phenolic compounds that aid in adaptation and survival (Lehari & Kumar, 2024). VA can also be formed through the metabolic degradation of lignin or the oxidation of high-molecular-weight polyphenols like ferulic acid (Hoque et al., 2016). The role of VA in plants is multifaceted, ranging from providing structural support via lignin incorporation to serving as a signaling molecule in stress-response networks (Jalal et al., 2025).

5.2 Physicochemical and Biological Properties

VA is a solid at room temperature with a melting point of 211.5 degrees C and a solubility of approximately 1.5 mg/ml in water at 14 degrees C. Its chemical structure allows it to insulate biological membranes and inhibit lipid peroxidation by scavenging hydroxyl radicals and lipid peroxy radicals (Tai et al., 2011). Beyond its role in plants, vanillic acid has attracted attention in pharmacological research for its anti-inflammatory, neuroprotective, and cardioprotective properties, many of which are linked to its ability to modulate signaling pathways such as NF-kappaB and Nrf2 (Calixto-Campos et al., 2015).

Table 2. Physicochemical and Biological Characteristics of Vanillic Acid

Parameter	Vanillic Acid (VA) Characteristics	Relevant Functional Roles
Chemical Name	4-hydroxy-3-methoxybenzoic acid (Parvin et al., 2024)	Precursor for vanillin and lignin synthesis (Ullah et al., 2021)
Biosynthetic Pathway	Shikimic/Phenylpropanoid pathway (Lehari & Kumar, 2024)	Upregulated under abiotic stress conditions (Parvin et al., 2024)
Antioxidant Action	Direct scavenging of OH. and O ₂ .- (Parvin et al., 2024)	Inhibition of lipid peroxidation and ROS damage (Rahman et al., 2024)
Pharmacological Properties	Anti-inflammatory, hepatoprotective, neuroprotective (Calixto-Campos et al., 2015)	Potential therapeutic use in metabolic syndrome (Ullah et al., 2021)

6. Exogenous Vanillic Acid as a Mitigation Strategy for Cadmium Toxicity

The exogenous application of vanillic acid has been identified as an effective means to enhance cadmium tolerance in various crops, providing a blueprint for its application in Brassica rapa. Foliar spray or root pretreatment with VA targets several critical physiological and biochemical bottlenecks induced by cadmium toxicity (Ullah et al., 2021).

6.1 Restoration of Morpho-Physiological Attributes

Studies in rice and tomato indicate that VA pretreatment significantly suppresses the cadmium-induced reduction in growth and biomass accumulation. In the context of Brassica, the application of VA (often at concentrations of 2 to 4 mM) helps maintain the chlorophyll content and the integrity of the photosynthetic machinery under metal stress (Rahman et al., 2024). VA prevents the degradation of pigments and supports stomatal conductance, ensuring that the plant can continue carbon fixation even in the presence of cadmium (Parvin et al., 2024). Furthermore, VA improves the water status of the plant, increasing leaf relative turgidity and alleviating the physiological drought symptoms associated with cadmium exposure (Mahmud et al., 2020).

6.2 Upregulation of the Antioxidant Defense System

The primary mechanism by which VA mitigates cadmium toxicity is the modulation of the plant's

antioxidant machinery. VA treatment leads to an increase in the activities of key antioxidant enzymes, including SOD, CAT, POD, and APX (Ahammed et al., 2021). These enzymes provide a first line of defense against the cadmium-induced ROS burst. Additionally, VA enhances the components of the ascorbate-glutathione (AsA-GSH) cycle, which is vital for maintaining cellular redox homeostasis (Li et al., 2022). By increasing the pool of non-enzymatic antioxidants such as ascorbic acid (AsA) and reduced glutathione (GSH), VA ensures that the plant has a high capacity for neutralizing H₂O₂ and other harmful oxidants (Kamran et al., 2021). This results in a significant reduction in MDA levels and relative membrane permeability, thereby preserving the structural integrity of the cell (Kaya et al., 2020).

6.3 Enhancement of MG Detoxification and Glyoxalase Enzymes

Vanillic acid also plays a critical role in the detoxification of methylglyoxal. Supplementation with VA under cadmium stress has been shown to increase the activities of Gly I and Gly II enzymes (Arif et al., 2020). This upregulation facilitates the rapid conversion of MG into D-lactate, reducing the risk of protein and DNA glycation. The maintenance of the GSH pool by VA is particularly important here, as GSH acts as a necessary cofactor for the Gly I enzyme (Hasanuzzaman et al., 2020). By strengthening both the antioxidant and glyoxalase systems, VA provides a comprehensive defense against the

secondary stresses induced by cadmium toxicity (Raza et al., 2021).

6.4 Osmotic Adjustment and Nutrient Homeostasis

Cadmium stress often leads to the loss of osmotic balance. VA application has been observed to stimulate the accumulation of compatible solutes like proline and soluble sugars. Proline not only serves as an osmoprotectant but also acts as an

antioxidant and a chaperone for stabilizing proteins and membranes (Saleem et al., 2022). Furthermore, VA influences ion regulation, helping to maintain the levels of essential nutrients like Ca²⁺, K⁺, and Mg²⁺ which are often displaced by cadmium ions. This restoration of nutrient homeostasis is crucial for maintaining enzymatic activities and structural integrity within the plant cell (Sun et al., 2025).

Table 3. Metabolic Responses to Cadmium and Vanillic Acid

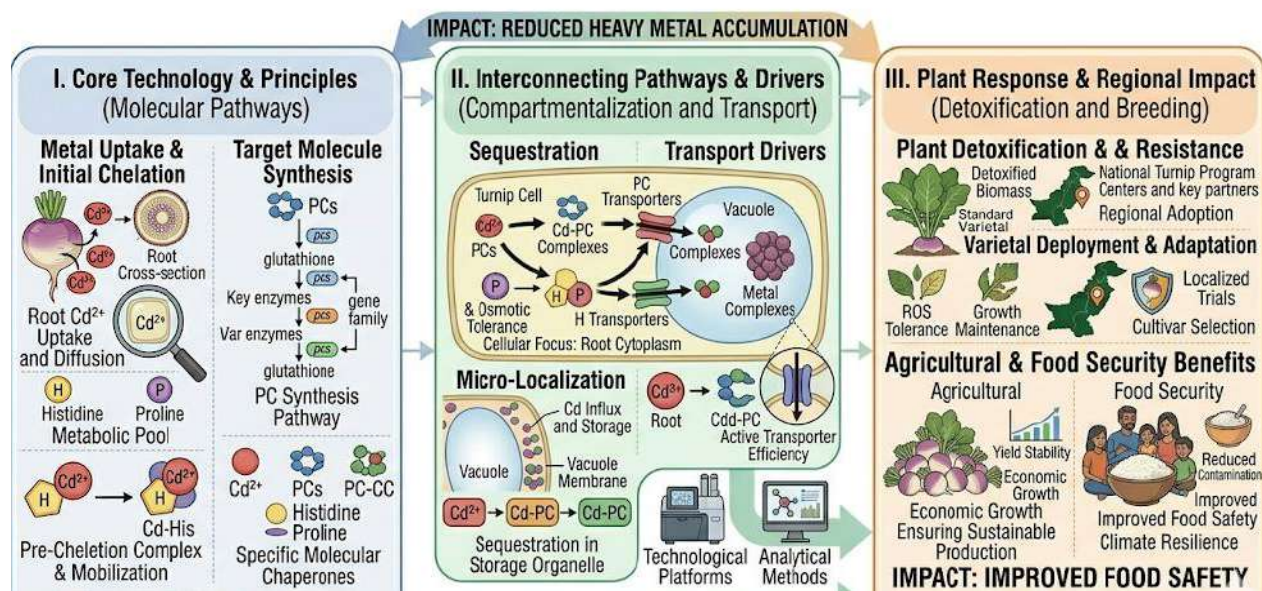
Metabolic Parameter	Effect of Cadmium	Effect of Exogenous VA
Hydrogen Peroxide (H ₂ O ₂)	Significant increase (Mahmud et al., 2020; Noor et al., 2024)	Significant reduction (Mahmud et al., 2020; Rahman et al., 2024)
Malondialdehyde (MDA)	Elevated levels (lipid peroxidation) (Hu et al., 2023; Mahmud et al., 2020)	Decreased levels (Mahmud et al., 2020; Rahman et al., 2024)
Glyoxalase I & II Activity	Potential inhibition (Mahmud et al., 2020)	Significant upregulation (Mahmud et al., 2020; Parvin et al., 2024)
Methylglyoxal (MG) Content	Accumulation (cytotoxicity) (Mahmud et al., 2020)	Accelerated detoxification (Mahmud et al., 2020; Parvin et al., 2024)
Proline Content	Stress-induced increase (Bisht & Garg, 2022; Parvin et al., 2024)	Further enhancement (protective) (Parvin et al., 2024; Rahman et al., 2024)

7. Metal Sequestration and the Role of Phytochelatins in Brassica rapa

The ability of Brassica rapa to withstand cadmium stress is intimately linked to its capacity for metal chelation and compartmentalization. One of the most significant responses to cadmium entry is the induction of phytochelatins

(PCs), which are cysteine-rich peptides synthesized from glutathione (Noor et al., 2024). Phytochelatins and amino acids play a central role in Cd detoxification by chelation and vacuolar sequestration, as depicted in Figure 2. VA treatment enhances this intrinsic detoxification pathway.

Figure 2: Mechanism of Metal Sequestration and Phytochelatin-Mediated Detoxification in Turnip



7.1 Chelation and Vacuolar Compartmentalization

Cadmium ions are chelated by phytochelatin to form stable Cd-PC complexes. These complexes are then actively transported into the vacuole, effectively isolating the toxic metal from sensitive cytosolic processes and organelles (Gupta et al., 2013). In turnips, this mechanism allows the plant to accumulate high concentrations of cadmium in its leaves (often exceeding 100 mg/kg) without showing signs of lethal injury (Anonymous, 2018). VA application has been shown to increase the PC content in cadmium-stressed plants, suggesting that VA enhances the plant's inherent capacity for metal sequestration (Bisht et al., 2023).

7.2 Role of Amino Acids as Chelating Agents

Beyond phytochelatin, certain amino acids play a critical role in cadmium detoxification. In *Brassica rapa*, cadmium stress induces a significant increase in the synthesis of free amino acids like histidine, proline, and aspartic acid (Khan et al., 2020). Histidine is thought to serve as a metal-chelating agent, similar to its role in nickel hyperaccumulators, while aspartic acid can form Cd-Asp complexes that reduce the

bioavailability of Cd²⁺ ions within the cell (Wani et al., 2022). These amino acids also serve as substrates for the synthesis of stress-responsive proteins and secondary metabolites like phenolics (Sindhushree et al., 2025).

8. Molecular Signaling and Genomic Responses to Vanillic Acid and Cadmium

The mitigation of cadmium toxicity by vanillic acid is not merely a biochemical buffering process but involves a complex orchestration of gene expression and signaling pathways. VA likely acts as a signaling molecule that triggers the activation of transcription factors and the regulation of metal transport proteins (Vijiyakumar & Prince, 2025).

8.1 Regulation of Heavy Metal Transporters

The transport of cadmium in *Brassica rapa* is mediated by several families of protein transporters, including P-type ATPases (HMAs), the Zinc-Iron Permease (ZIP) family, and the Natural Resistance-Associated Macrophage Protein (NRAMP) family (Tao & Lu, 2022). Cadmium stress typically upregulates genes such as *BcIRT1* and *BcNramp1* in the roots, facilitating the influx of Cd²⁺ ions (Zhang et al., 2024).

Phytoprotectants like VA may work by modulating the expression of these genes. For instance, VA application can downregulate the expression of uptake transporters while upregulating efflux proteins or vacuolar membrane transporters (like ABC transporters) to enhance compartmentalization efficiency (Khan et al., 2024). This molecular "gating" helps maintain a lower concentration of free cadmium in the cytoplasm, thereby protecting vital metabolic enzymes (Požgajová et al., 2022).

8.2 Integration with Hormone Signaling Pathways

The plant's response to cadmium and VA is also mediated through cross-talk with hormone signaling pathways. Cadmium stress is known to trigger the production of signaling molecules like hydrogen sulfide (H₂S), nitric oxide (NO), and various phytohormones such as abscisic acid (ABA), salicylic acid (SA), and ethylene (Kumar et al., 2024). VA application has been shown to modulate the synthesis and levels of these hormones, which in turn regulate adaptive physiological changes like stomatal closure and the induction of root adaptations (Zhang et al., 2019).

For example, the induction of the phenylpropanoid pathway by VA leads to the synthesis of other phenolic compounds and lignin, which can strengthen the cell wall and provide a physical barrier against metal entry. This structural reinforcement is a critical aspect of cadmium avoidance and tolerance in Brassica species (Beyer, 2025).

9. Comparative Insights: Vanillic Acid vs. Other Mitigation Strategies

While vanillic acid is a powerful phytoprotectant, its efficacy in Brassica rapa should be viewed in the context of other available mitigation strategies, such as the use of essential nutrients, other organic acids, or soil amendments (Singh et al., 2016).

9.1 Nutrients and Other Organic Acids

The application of calcium (Ca²⁺) or silicon (Si) has been shown to attenuate cadmium-induced oxidative stress in Brassica by modulating the AsA-GSH pathway and decreasing cadmium accumulation (Bisht & Garg, 2022). Similarly, organic acids like citric acid can enhance cadmium tolerance and phytoremediation efficiency by improving metal chelation and the glyoxalase system. Salicylic acid, another phenolic derivative, also plays a key role in protecting plants from heavy metal stress through the activation of antioxidant enzymes (Sindhusree et al., 2025). Vanillic acid's advantage lies in its high antioxidant potential and its role as a natural metabolite that is easily integrated into the plant's own secondary metabolic pathways (Vitelli et al., 2024).

9.2 Soil Amendments: Biochar and Selenium

The integration of biochar into the soil provides a physical and chemical remediation strategy that complements the biological action of VA. Biochar can immobilize cadmium in the soil through adsorption and pH elevation, thereby reducing the initial load of metal that the plant must contend with (Hu et al., 2023). Selenium (Se) and Se-nanoparticles have also emerged as potent mitigators, working synergistically with compounds like melatonin or VA to enhance genomic stability and reduce cadmium uptake (Noor et al., 2024).

Table 4. Comparison of Mitigation Agents Against Cadmium Toxicity

Mitigation Agent	Primary Mechanism of Action	Specific Impact on Cadmium
Vanillic Acid (VA)	Antioxidant/Glyoxalase modulation (Mahmud et al., 2020)	Enhances internal tolerance and chelation (Parvin et al., 2024)
Biochar (BC)	Soil immobilization and pH regulation (Hu et al., 2023)	Reduces soil bioavailability and plant uptake (Hu et al., 2023)

Selenium (Se)	Formation of insoluble CdSe; ROS regulation (Noor et al., 2024)	Minimizes Cd content in crops (Noor et al., 2024)
Citric Acid (CA)	Metal chelation and phytoextraction aid (Mahmud et al., 2018)	Improves biomass and metal accumulation (Mahmud et al., 2018)

10. Practical Implications for Phytoremediation and Food Security

The use of vanillic acid in Brassica rapa cultivation has dual implications depending on the ultimate goal: whether to produce safe food or to remediate contaminated soil (Javed et al., 2025).

10.1 Optimizing Phytoextraction

For phytoremediation purposes, the goal is to maximize the accumulation of cadmium in the harvestable parts of the plant. VA application can facilitate this by increasing the plant's biomass and its translocation factor (TF), allowing more cadmium to be moved from the roots to the shoots without killing the plant (Zulfiqar et al., 2022). Since turnips are high-biomass producers, enhancing their tolerance through VA can significantly increase the "phytoextraction rate" (total amount of metal removed per hectare) (Ghadge & Trivedi, 2026).

10.2 Ensuring Food Safety and Yield

In agricultural settings where the goal is food production, VA serves as a safeguard. By reducing oxidative stress and maintaining the photosynthetic rate, VA prevents the yield losses that typically occur in cadmium-polluted soils (Kumari et al., 2022). Furthermore, by promoting the sequestration of cadmium in stable, non-toxic forms within the plant or by potentially reducing root-to-shoot translocation in certain cultivars, VA can help keep the concentration of bioavailable cadmium in the edible parts below the permissible limits set by health organizations like the WHO (Smith et al., 2023).

11. Second-Order Insights and Future Directions in VA-Mediated Mitigation

The research surrounding vanillic acid and cadmium in Brassica rapa points toward several emerging themes that could redefine our approach to environmental management (Chamoli et al., 2026).

11.1 Metabolic Reprogramming and Stress Memory

Exogenous VA application may induce a form of "stress memory" or priming in the plant. By activating the antioxidant and glyoxalase systems prior to or during the onset of cadmium stress, VA prepares the cellular environment to handle the influx of metal ions more efficiently (Raza et al., 2020). This metabolic reprogramming shifts the plant's focus from rapid growth to a more resilient, defense-oriented state. Future research should investigate the duration of this priming effect and whether it can be inherited by subsequent generations (Shahid et al., 2017).

11.2 Synergies with Rhizosphere Microbiota

A crucial but under-explored area is the interaction between VA and the rhizosphere microbial community. Phenolic acids are known to influence root exudation patterns and can attract beneficial microorganisms like PGPR and AMF (Unsal et al., 2020). These microbes can further assist in cadmium detoxification by producing siderophores, sequestering metals in their own biomass, or altering the chemical speciation of metals in the soil (Kumar et al., 2024).

11.3 Nano-Formulations and Targeted Delivery

The effectiveness of VA can be limited by its solubility and environmental degradation. The development of nano-encapsulated vanillic acid or VA-functionalized nanoparticles (similar to the melatonin-selenium nanocomposites) could provide a more controlled and targeted delivery system (Beyer, 2025). This would allow for lower application rates and higher efficiency in delivering the phytoprotectant to the site of stress within the plant (Jung et al., 2021).

11.4 Genetic Engineering of the VA Pathway

Finally, understanding the genes involved in the endogenous synthesis of VA provides a path for genetic engineering. By overexpressing the genes of the phenylpropanoid pathway that lead to vanillic acid production, researchers could develop "self-mitigating" Brassica rapa cultivars that are naturally more tolerant to cadmium and other heavy metals (Parmar et al., 2020). This would eliminate the need for repeated exogenous applications and provide a more permanent solution for farming on contaminated lands (Haider et al., 2021).

12. Conclusions

Cadmium-induced toxicity in turnip (*Brassica rapa*) manifests through multifaceted disruptions encompassing reduced biomass, impaired photosynthesis, oxidative burst, lipid peroxidation, methylglyoxal accumulation, and nutrient imbalances, ultimately threatening yield and food safety in contaminated agricultural systems. Exogenous application of vanillic acid effectively alleviates these adverse effects by acting as a potent antioxidant and signaling molecule that restores morpho-physiological attributes, upregulates the enzymatic and non-enzymatic antioxidant defense systems (SOD, CAT, APX, AsA-GSH cycle), enhances glyoxalase-mediated detoxification of cytotoxic MG, promotes osmoprotectant accumulation (proline), and strengthens metal chelation via phytochelatin and vacuolar sequestration. By modulating heavy metal transporters and integrating with phytohormone and secondary metabolic pathways, VA not only improves internal Cd tolerance but also supports safer edible tissue production while potentially enhancing phytoremediation efficiency through increased biomass and translocation. Compared to soil amendments or other organic acids, VA provides a targeted, plant-compatible mitigation strategy with low environmental risk. To maximize its practical utility, future research should focus on optimizing application protocols, developing nano-encapsulated formulations for efficient delivery, elucidating long-term stress memory and transgenerational effects, and engineering

Brassica cultivars with enhanced endogenous VA biosynthesis. Integrating VA application with beneficial rhizosphere microbes and complementary amendments could further amplify resilience. Ultimately, vanillic acid represents a promising, sustainable biotechnological tool for mitigating heavy metal stress, safeguarding crop productivity, and ensuring food security in cadmium-polluted agroecosystems worldwide.

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