JEE Journal of Ecological Engineering

Journal of Ecological Engineering, 2025, 26(8), 451–462
https://doi.org/10.12911/22998993/204352
ISSN 2299–8993, License CC-BY 4.0

Received: 2025.03.19
Accepted: 2025.05.26
Published: 2025.06.10

Exogenous salicylic acid enhances physiological responses, nutrient uptake by metabolic adjustments to alleviate cadmium stress in rapeseed (*Brassica napus* L.) cultivars

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ABSTRACT

Cadmium (Cd) is considered as one of the highly toxic and non-essential heavy metal, having no biological functions, and inhibit plant growth and development, owing serious threat to crop productivity. Morphophysio-biochemical attributes affected by bioaccumulation in the plant tissues causes phytotocicity. A promising sustainable strategies to ameliorate the cadmium stress in *Brassica napus* (L.) by salicylic acid remain unrevealed. Two varieties (Super Canola, Shiralee) were grown in pots, while cadmium chloride (400 µm) was randomly applied three times and two levels of salicylic acid (SA) (0.25 mM) were used. Plants exposed to Cd stress significantly decreased the morpho-physiological attributes, photosynthetic pigments and minerals uptake. Results revealed that the foliar application of SA to cadmium affected plants showed significant improvement in shoot length (6%) root length (10%), shoot fresh (32%) shoot dry weights (32%), root fresh (28%) and root dry weights (35%) compared to Cd plants. Moreover, SA application showed decline in H_2O_2 and malondialdehyde (MDA) contents through regulating the antioxidant activities (POD 60%, SOD 28%, CAT 43%). Similarly, both varieties showed recovery with SA, improved photosynthetic pigments and nutrient uptake. In conclusion, It was revealed that cadmium (Cd) significantly affects the morphological, physiological, and biochemical characteristics of plants, and can be mitigated through the application of salicylic acid (SA) for better performance. Salicylic acid was particularly effective in alleviating the negative impacts of cadmium stress on *Brassica napus* varieties. Overall, this research indicates that salicylic acid effective role in ameliorating stress conditions and contributes to the improvement of plant profile.

Keywords: salicylic acid, *Brassica napus*, cadmium stress, reactive oxygen species, antioxidant activities

INTRODUCTION

Rapeseed (*Brassica napus* L.) is an herbaceous biennial plant belonging to the *Cruciferae* family. On the other hand, it has edible foliage and roots. Additionally, the extracted glucosinolates from this plant have great medical significance in treating many cancers (Paul *et al*., 2019). The rapeseed (*Brassica napus* L.) is a widely grown horticulture crop. Cooking oil and biofuel are produced from the seeds of this plant (Cartea *et al*., 2019). *B. napus* is key oilseed crop in Pakistan which contributes 15% of the total oil production from oilseed crops. It is widely cultivated in the Punjab and Khyber Pakhtunkhwa provinces of Pakistan (Wolko *et al*., 2022). Young leaves are used as green vegetables because they are rich in vitamin A, vitamin C, and iron. In addition, brassica roots are rich in vitamins, minerals, and roughage, which are crucial for optimum health. Brassica bulb pulp has no cholesterol, 34 mg of calories, 2.2% fiber, 7.84% carbohydrates, 1.10% protein, and 0.12% fat per 100 g (Singh *et al*., 2022).

Heavy metals pose significant risks to ecosystems and human health by degrading soil quality, impairing plant development, and disrupting photosynthetic processes (Bharti and Sharma, 2022). With the global population projected to reach 9 billion by 2050, ensuring food security amid rising agricultural challenges remains critical (Ray *et al*., 2013). Cadmium, a persistent environmental pollutant, severely threatens crop productivity and plant survival by inducing cellular toxicity (Sinha *et al*., 2021). Recognized as a carcinogen by the World Health Organization, Cd contaminates soil, water, and food chains, endangering human and animal health (Chen *et al*., 2019). Its rapid plant absorption triggers phytotoxic effects, disrupts nutrient assimilation, and reduces crop yield and quality, ultimately leading to plant mortality (Haider *et al*., 2021). Many studies have examined the phytotoxic effect of Cd on various plant species and in one reported study among them on B. napus found that it impaired plant growth and yield, altered biochemical pathways, reduced photosynthetic pigments, and hampered various enzymatic pathways (Ahmed *et al*., 2023).

This study examines Cd's multifaceted impact on plant physiology, particularly its disruption of redox balance through oxidative stress a condition marked by excessive reactive oxygen species (ROS) production overwhelming antioxidant defenses (El Rasafi *et al*., 2022). ROS accumulation under Cd stress damages cellular structures, including lipids, proteins, and DNA, impairing growth and development. To counteract this, plants activate antioxidant systems to neutralize ROS and restore redox equilibrium (Lu *et al*., 2018). Cd further destabilizes cell membranes by binding to sulfhydryl groups in proteins and promoting lipid peroxidation (Naismi *et al*., 2023).

Antioxidant mechanisms are essential for plant resilience under heavy metal stress. Cd exposure compromises these systems by reducing antioxidant reserves and inhibiting enzymes like superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (El Rasafi *et al*., 2022). This investigation evaluates Cd's influence on antioxidant enzyme activity and non-enzymatic compounds, such as ascorbate and carotenoids. Photosynthesis, a process susceptible to environmental stressors, is also disrupted by Cd through chlorophyll degradation and thylakoid membrane damage, directly affecting plant productivity (Saini and Dhania, 2020; Bharti and Sharma, 2022).

Nutrient homeostasis is another casualty of Cd toxicity, as it interferes with the uptake of essential elements like nitrogen, phosphorus, and potassium, exacerbating plant vulnerability (Nazar *et al*., 2012). Plants counteract Cd stress through hormonal signaling pathways and the synthesis of protective metabolites, which activate defense responses. These adaptations highlight the need to unravel molecular mechanisms underlying Cd tolerance to develop strategies for sustainable agriculture and food security. Salicylic acid (SA), a naturally occurring phytohormone, has gained attention for its ability to alleviate cadmium (Cd) toxicity in plants. SA is central to mediating defense responses against biotic and abiotic stressors while modulating key physiological processes such as growth regulation and environmental adaptation (Ray *et al*., 2013). Functioning as a signaling molecule, SA influences photosynthesis and enhances antioxidant activity, enabling plants to counteract stress-induced damage (Peng *et al*., 2021). Exogenous SA application has proven effective in bolstering plant resilience to Cd toxicity by improving cellular redox balance and stress-responsive pathways (Ngou *et al*., 2022). Studies indicate that SA mitigates Cd-triggered oxidative stress by upregulating genes associated with ROS scavenging, thereby strengthening cellular defense mechanisms (Li *et al*., 2019).

This study investigates the impact of Cd stress on several morpho-physiological and biochemical attributes in *Brassica napus* cultivars and explores the potential of SA as a mitigation strategy. The objectives of this research are to: (1) assess the impact of Cd stress on the morpho-physio-biochemical attributes of *Brassica napus*; (2) evaluate the effectiveness of SA in ameliorating the adverse effects of Cd stress; (3) investigate the effects of Cd on photosynthetic pigments and photosynthesis efficiency under Cd stress with and without SA application; and (4) evaluate the impact of Cd on nutrient uptake. Furthermore, the findings of this study are expected to provide valuable insights into the mechanisms of Cd toxicity in *Brassica napus* and the potential of SA as a stress mitigation strategy. This knowledge is crucial for developing sustainable agricultural practices that can enhance plant resilience against Cd stress, thereby contributing to food security and human health. The findings of this study provide a comprehensive understanding of the effects of Cd on plant cells, including its impact on antioxidant systems, nutrient uptake, and photosynthetic pigments. This knowledge is crucial for developing strategies to mitigate the adverse effects of Cd on plant health and agricultural productivity.

MATERIALS AND METHODS

This pot experiment was conducted in Old Botanical Garden, Department of Botany, University of Agriculture Faisalabad. Seeds of mustard plant (*Brassica napus*) obtained from Ayub Agriculture Research Institute (AARI) Faisalabad. During the experiment period, climatic conditions were average Photosynthetic Active Radiation (PAR 1000 to 2000 μmol m^{-2} s^{-1}), minimum and maximum relative humidity (40% and 70%) average temperature, and rainfall were 15 °C to 25 °C and 400 to 600 mm, respectively. Seeds were sown 1-inch depth in the pot containing 10 kg of sandy-loamy soil. Two cultivars of (*B. napus)* were used for this study such as Super Canola and Shiralee. Stress of cadmium chloride (400 μm) was applied three times after seven days interval, as well as two levels of salicylic acid (0.25 mM), were used. Two weeks after germination, four healthy plants were left per pot by thinning. Plants were harvested to record different morpho-physiological and biochemical attributes. Two plants were left in each pot for maturity to record the yield attributes. The experiment was performed in a completely randomized design with three replications of each treatment.

Growth attributes

Mustard plants were harvested at the flowering stage to record shoot-root lengths and fresh weights. Shoot and root lengths were measured with a standard ruler. Fresh biomass samples were oven-dried at 75°C for four days to determine dry weights.

Photosynthetic pigments

Chlorophyll and carotenoid levels in fresh leaves were quantified following Arnon's method (1949). Fresh leaf tissue (0.1 g) was finely chopped, immersed in 5 mL of 80% acetone, and left overnight at room temperature. The absorbance of the supernatant was measured at 645, 663, and 480 nm using a spectrophotometer. The Equations (1–3) were used as follows:

$$\text{Chl. a (mg/g)} = [12.7(\text{OD663}) - 2.69(\text{OD645})] \times V/(1000 \times W) \quad (1)$$

$$\text{Chl. b (mg/g)} = [22.9(\text{OD645}) - 4.68(\text{OD663})] \times V/(1000 \times W) \quad (2)$$

$$\text{Carotenoids} = [\text{OD663} - 0.638(\text{OD645})] \times V/(1000 \times W) \quad (3)$$

where: V – extract volume [mL]; W – leaf fresh weight [g].

Total soluble protein and free amino acids

Soluble proteins were analyzed via Bradford assay (1976). Fresh leaves (0.25 g) were homogenized in 5 mL pH 7.8 buffer, centrifuged at 12,000 rpm (4 °C, 15 min), and mixed with Bradford reagent. Absorbance was read at 595 nm. Free amino acids were measured using Hamilton's protocol (1973): 0.5 mL extract was mixed with ninhydrin (2%) and pyridine (10%), heated (100 °C, 40 min), diluted to 25 mL, and analyzed at 570 nm.

Flavonoids and ascorbic acid

Flavonoid content was determined as per Zhishen et al. (1999). Leaf tissue (0.1 g) was homogenized in 80% acetone, centrifuged, and supernatant absorbance recorded at 510 nm. Ascorbic acid was extracted from fresh leaves (0.1 g) in 6% trichloroacetic acid and quantified at 530 nm.

Hydrogen peroxide and malondialdehyde

Hydrogen peroxide (H_2O_2) concentration was quantified after Velikova et al. (2000). Supernatant (1 mL) was mixed with phosphate buffer and KI solution (16.6 g/100 mL), vortexed, and measured at 590 nm. Malondialdehyde (MDA) content was assessed by Cakmak and Horst's (1991). Supernatant (1 mL) was reacted with 0.5% TBA in 20% TCA, heated (95°C, 15 min), cooled, and absorbance recorded at 532 nm and 600 nm.

Antioxidant enzyme assays

SOD was assayed in enzyme extracts (0.1 g leaves in 50 mM phosphate buffer + 1 mM DTT) reacted with NBT, EDTA, methionine, and riboflavin (Dixit et al., 2001). POD activity was measured via guaiacol-H_2O_2 oxidation (Mukherjee and Choudhuri, 1983). CAT activity was determined by H_2O_2 decomposition at 240 nm (Aebi, 1984).

Statistical analysis

The experiment followed a completely randomized design with triplicate treatments. Data variance was analyzed (ANOVA) using Statistix 8.1, with mean differences assessed via Fisher's test ($p < 0.05$). Graphs were plotted in GraphPad Prism; PCA and correlation analyses were performed in RStudio.

RESULTS

Growth attributes

Plant morphological attributes have significance importance against survival in heavy metal. To find out the mitigation impact of salicylic acid in two varieties of Mustard (*Brassica napus* L.) cadmium stress was applied with control and 400 µM concentrations, fresh shoot, root, dry shoot, dry root, shoot length and root length were recorded (Figure 1a). Current results expressed significant difference in growth related attributes in mustard plants under cadmium and control conditions. Growth attributes were significantly eased in cadmium stress while salicylic acid minimized the cadmium toxicity. Cadmium stress caused 15% reduction in shoot fresh weight of brassica varieties as compared to control while salicylic acid foliar spray significantly this attribute up to 32%. Brassica varieties showed 21% decline in their root fresh weights as compared to their control, Salicylic acid declined this deduction up to 28% in varieties of *Brassica napus* (Figure 1b). Sharilee variety showed more pronounced results for shoot fresh weight and root fresh weight with 0.25 mM foliar application of salicylic acid. Cadmium toxicity had 15% and 22% destruction for shoot and root dry weights respectively. While foliar application of 0.25 mM salicylic acid assisted 32% and 35% positive impact on mentioned attributes of Brassica varieties (Figure 1c). Plant shoot length and root length under stress environment depleted up to 23% and 19% in Brassica genus while exogenous application of salicylic acid overthrown this deduction 6% and 10% for this attribute (Figure 1c,d).

Photosynthetic activity

Foliar effect of salicylic acid on growth parameters of canola varieties under cadmium stress. Physiological activities were significantly reduced by the cadmium toxicity in both varieties, however exogenous application of salicylic acid mitigated the destructive response in Brassica varieties. The highest enhance was observed in super canola when 0.25 mM salicylic acid with no cadmium was applied. Chromium caused 12% decrease in chlorophyll *a* while salicylic acid 9% overcame this effect. For chlorophyll *b* 0.25 mM salicylic acid showed 12% positive impact as compared to control with no foliar and cadmium stress. Cadmium stress caused 37% reduction in chlorophyll b while the Salicylic acid minimized this destruction up to 9% (Figure 2a). However, Sharilee variety performed better for this attribute. Total chlorophyll reduction was recorded (37% and 35%) in V1 and V2 accordingly, foliar application of SA overcame the toxic effect of by 9% in V1 and 21% in V2 A protruding lessing 57% was observed for carotenoid when exposed to 400 µM cadmium stress. Salicylic acid foliar application caused 23% remarkable improvement as compared to control of foliar with only stress. Sharilee's cultivar showed better performance for the carotenoid content under chromium stress (Figure 2b).

Reactive oxygen species

The accumulation of ROS, particularly hydrogen peroxide (H_2O_2) can result in oxidative stress. Excessive ROS can damage cellular components including lipids proteins and DNA through oxidation leading to cellular dysfunction and even cell death ultimately a deleterious impact on the physiological and morphological attributes of plants. Cadmium stress disturbed the plant metabolism and production of reactive oxygen species increased 34% in canola varieties. Most destruction was observed in Super Canola when exposed to 400 µM cadmium with no foliar application of salicylic acid (Figure 3a). Low concentrations of ROS that is of prime importance in plants for normal physiology other major process related to growth are 17% achieved as compared to toxicity effects of stress. Other ROS, MDA, contents were much increased by 12% in Super Canola at 400 µM CdCl2 treatment, in the current study 0.25 mM salicylic acid plant growth regulator reduced these harmful effects 5% (Figure 3b). Similarly Super Canola shows better performance for the lessen accumulation of reactive oxygen species.

Minerals ions

Mineral quantification for both varieties of Brassica varied when exposed to 400 µM cadmium stress. Minimum Ca^{2+} content in root and leaf was observed in Super Canola when (400 µM + No SA spray) conditioned applied. However, cadmium stress caused 15% and

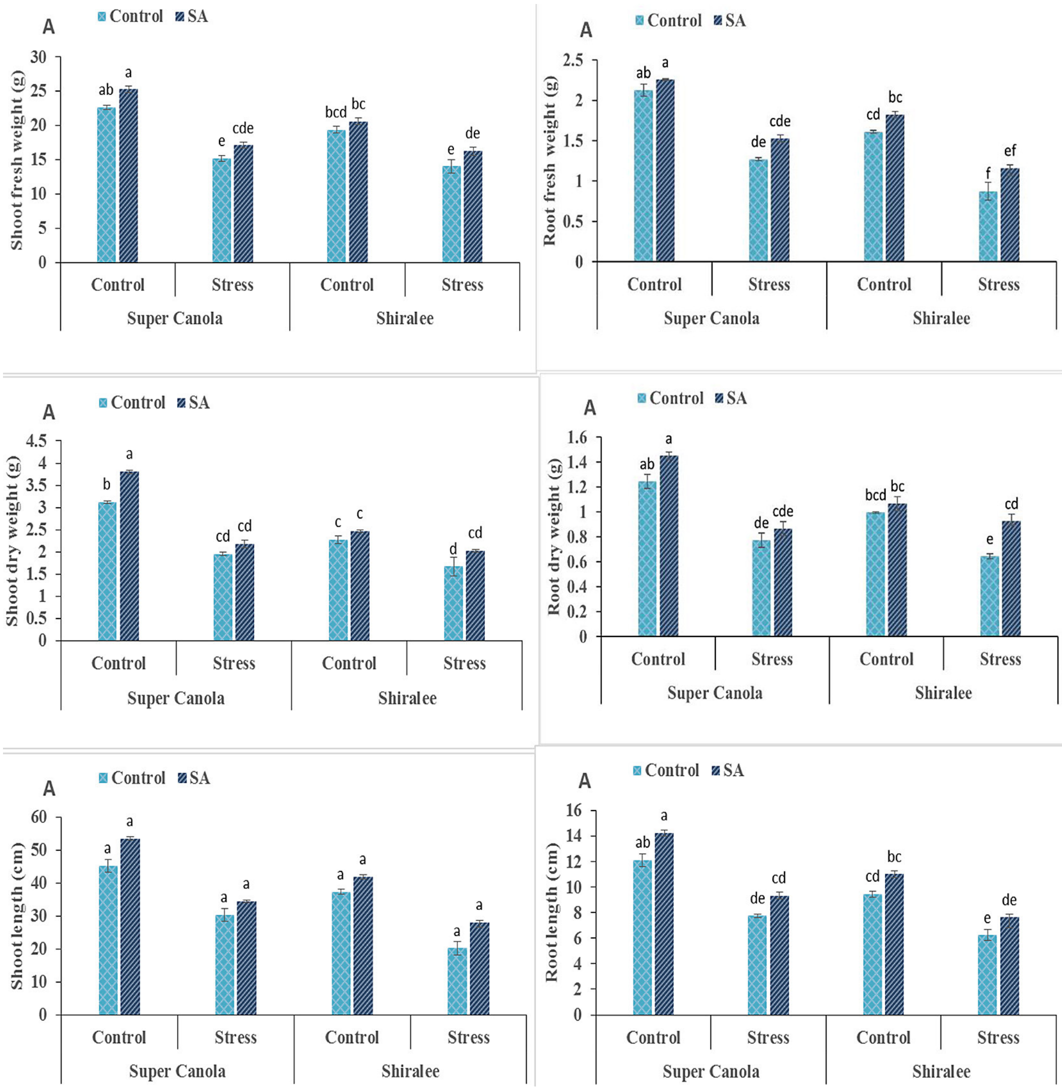

Figure 1. Fresh and dry root, shoot weight, and length of root and shoot of rapeseed (*Brassica napus* L.) plants when SA is foliarly applied under cadmium stress conditions

20% reduction in root and shoot of Ca^{2+} ions for both varieties of *Brassica napus* L. salicylic acid alleviated these affects by 2% and 14% respectively (Figure 4a). Contents of K^{+} ions were suppressed 24% and 9% respectively in root and leaf of Brassica genus under cadmium stress, while foliar applied SA enhanced 12% and 5% ion concentrations from rusticity of cadmium (Figure 4b). Cadmium caused most prime decrease in sodium ion from whole ionic chart. Sodium ion showed 47% and 17% decrease ratio in root and leaf of both varieties, while foliar spray of SA (0.25 mM) reduced this toxicity by 17% and 24% respectively. Overall, Sharilee genotype shows better performance for the ionic content when applied with cadmium stress (400 μM) and 0.25 mM foliar application of salicylic acid (Figure 4b).

Enzymatic antioxidants

Antioxidant enzymes such as SOD, POD, and CAT are known to substantially reduce the levels of superoxide and hydrogen peroxide in plants. Peroxidase concentration was increased 16% when applied with 400 μM cadmium, meanwhile, foliar application of 0.25 mM salicylic acid significantly boosted this level up to 60% for the POD (Figure 5a). Salicylic acid a growth regulator of plants with concentration of 0.25mM enhanced the superoxide dismutase 28% (Figure5b) while under stress increase was only 7% respectively without foliar application of salicylic acid (No spray+ 400 μM $CdCl_2$). Similarly Super Canola shows better performance for the accumulation of enzymatic antioxidants. Activity of Catalase was

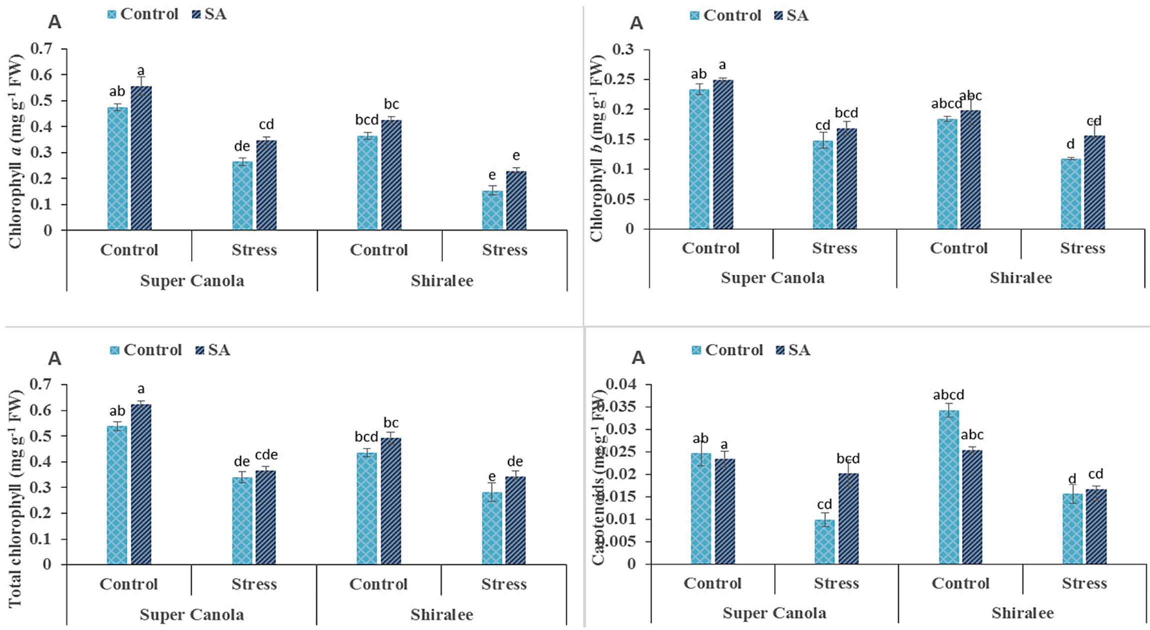

Figure 2. Chlorophyll *a*, *b*, total chlorophyll, and carotenoids of rapeseed (*Brassica napus* L.) plants when SA is foliarly applied under cadmium stress conditions

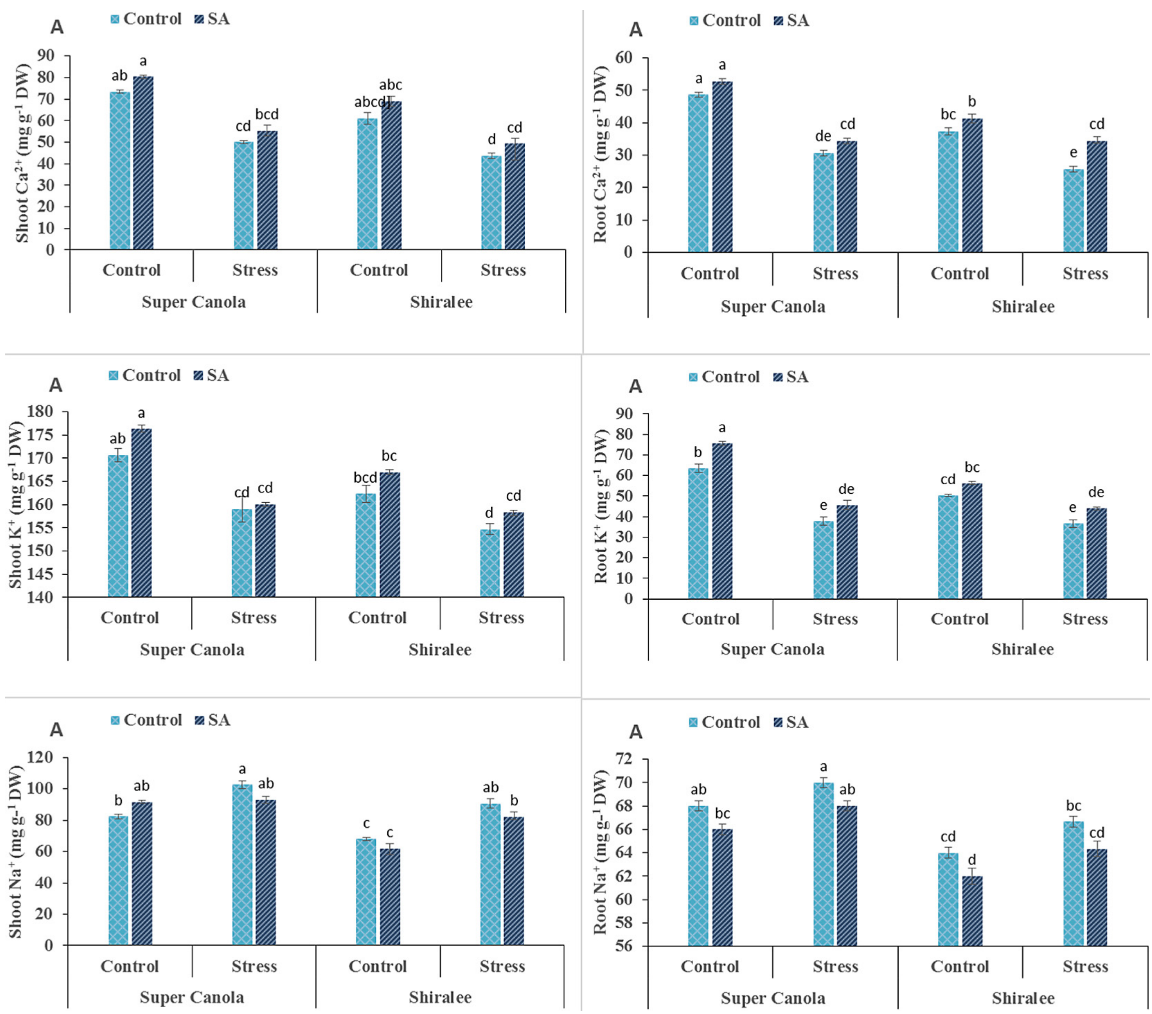

Figure 3. Leaf and root Ca^{2+}, leaf K^+ and Na^+ of rapeseed (*Brassica napus* L.) plants when SA is foliarly applied under cadmium stress conditions

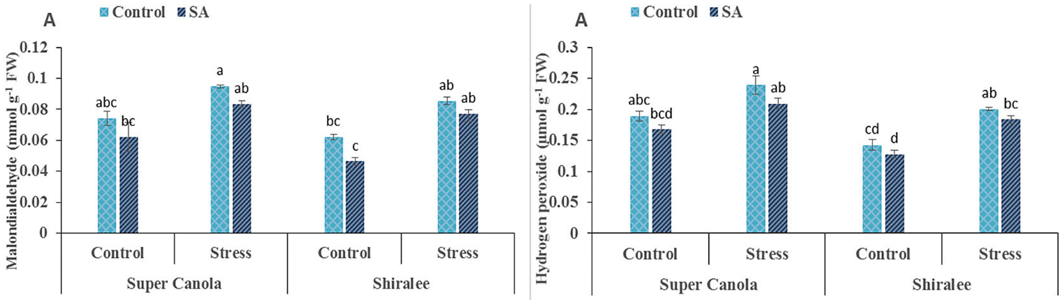

Figure 4. MDA and H_2O_2 of rapeseed (*Brassica napus*L.) plants when SA is foliarly applied under cadmium stress conditions

increased by the exposure of stress at 42% and 16% in V1 and V2, salicylic acid further enhanced their performance by 18% and 16% as compared to control conditions.

Non-enzymatic antioxidants

In both cultivar ascorbic acid contents, flavonoids contents were unregulated into cadmium stress. Maximum contents of flavonoids 44 % in V2 and 41% in V1 were recorded when treated with 400 µM cadmium chloride and salicylic acid foliar further boosting up the mentioned flavonoids contents (15% and 44%) in V1 and V2 respectively. and ascorbic acid contents (93 and 92%) were recorded in V_1 and V_2 respectively under salinity imposition rather than control. Similarly, an increase was also observed in ascorbic acid of V_1 and V_2 (22% and 29%) and further increased recorded by the application of salicylic acid was (9% and 14%) in V_1 and $V_{2.}$ Similarly, it was also found that amino acid and total soluble proteins (TSP) were enhanced in plants subjected to cadmium stress conditions. The results indicated increased amount of total amino acids (15% and 14%) and TSP (28% and 20%) in V_1 and V_2 correspondingly than non-stressed plants. These parameters were further improved by the SA application. Maximum production of total amino acids (13% and 12%) and TSP (30% and 9%) in V_1 and V_2 respectively.

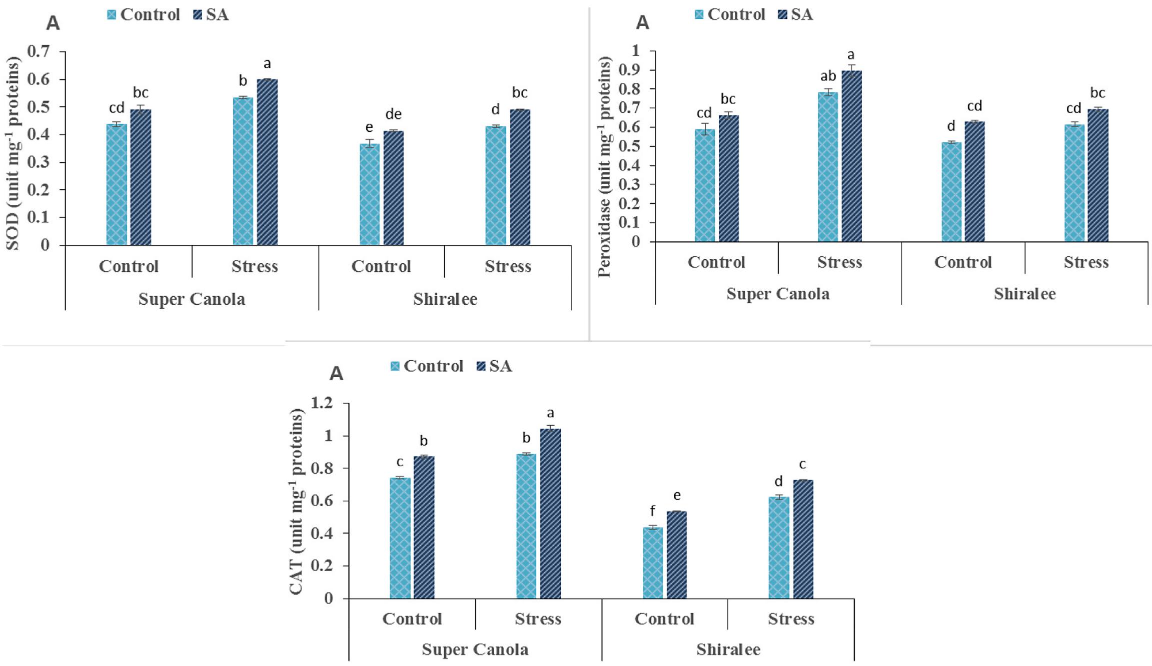

Figure 5. SOD, POD and CAT of rapeseed (*Brassica napus* L.) plants when SA is foliarly applied under cadmium stress conditions

DISCUSSION

The results of this study highlight the multifaceted impacts of cadmium (Cd) stress on plant physiology, with significant effects on morphological parameters, photosynthesis, ROS, antioxidant systems levels and inorganic ions. These findings underscore the complexity of Cd toxicity and the need for a comprehensive approach to understand and mitigate its effects. Morphological parameters including plant shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, shoot length, and root length decreased under cadmium stress in both varieties of turnip while the application of salicylic acid mitigates harmful effects of cadmium stress. Similar results have been observed in peppermint (Ahmad *et al*., 2018), wheat (Hussain *et al*., 2019), tomato (Alyemeni *et al*., 2018) and other plants under Cd stress. Cd-treated plants' decreased growth and biomass might be due to Cd's interference with cell cycle progression and irreversible alteration of the proton pumps, which disrupts membrane permeability (Liu *et al*., 2015). Reduced N, P, and K contents indicate that the Cd-induced membrane permeability disruptions restricted ion absorption, which could have hindered plant growth (Wang *et al*., 2019). Under Cd stress, SA induces

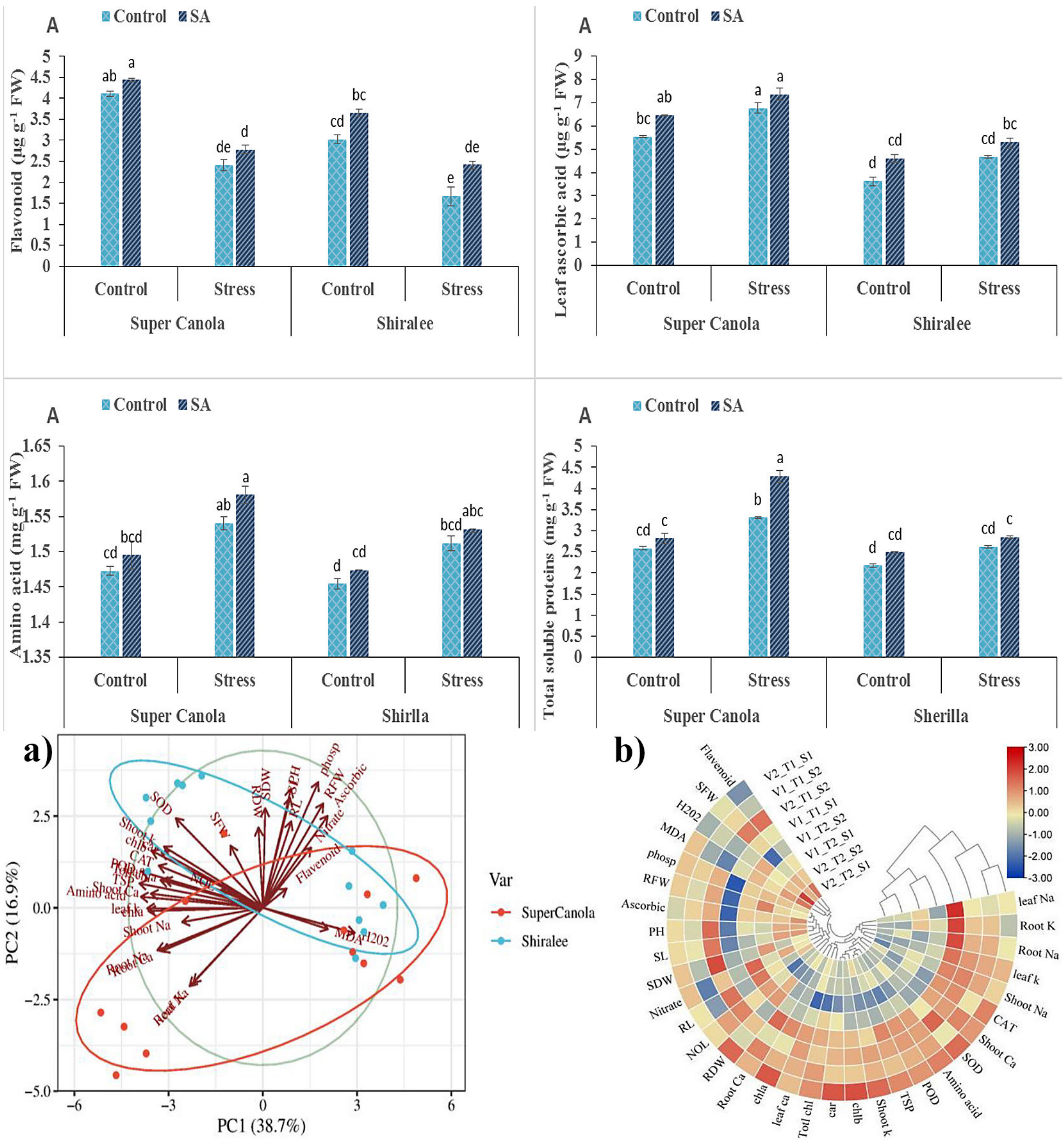

Figure 6. Flavonoids, ASA, TSP and Amino Acids of rapeseed (*Brassica napus* L.) plants when SA is foliarly applied under cadmium stress conditions: a) Principal component analysis (PCA) Biplot of physiological and biochemical traits in canola varieties under different conditions, b) Clustered heatmap representation of physiological and biochemical traits under different treatments: A circular visualization in R

gene expression and maintains optimal levels of endogenous SA, which enhances the Cd-induced growth inhibition Cd also decreases ROS levels under heavy metal stress (Zhang *et al*., 2015). The morphological changes observed in plants under Cd stress, including reduced growth and altered leaf morphology, are consistent with the physiological and biochemical effects of Cd. These changes are likely the result of the combined effects of reduced photosynthesis, nutrient deficiencies, oxidative stress, and altered antioxidant systems (Shah *et al*., 2020).

Photosynthesis, the fundamental process by which plants convert light energy into chemical energy, is particularly sensitive to environmental stresses. The observed reduction in photosynthetic efficiency under Cd stress suggests that Cd may interfere with the photosynthetic machinery, potentially through the alteration of photosynthetic pigments or the inhibition of photosynthetic enzymes. This impairment could have long-term implications for plant growth and productivity. In current experiment, cadmium stress caused significant decrease in content of chlorophyll *a*, *b*, carotenoid and total chlorophyll in both varieties of Brassica. Similar findings were shown by (Nazir *et al*., 2022) in milk thistle. According to (Lysenko *et al*., 2018) the accumulation of Cd^{2+} ions in the thylakoids and stroma of the chloroplasts as well as the substitution of Mg^{2+} bound into the chlorophyll molecule are the causes of the Cd-induced decreased of chlorophyll biosynthesis. By targeting different electron transport chain components in carboxylation processes coupled with PSII, the application of Cd^{+2} concentrations lowers the rate of photosynthesis (Shah *et al*., 2020).

The antioxidant systems are vital for plants to cope with environmental stresses, including heavy metal toxicity. The observed decrease in antioxidant levels and enzyme activities under Cd stress suggests that Cd may impair the antioxidant defense system, leading to oxidative stress (Talabany *et al*., 2023; Mostofa *et al*., 2019). This could explain the observed damage to cellular components and the reduced growth of plants under Cd stress. Enzymatic antioxidants like super oxide dismutase (SOD) and peroxidase (POD) showed significant results in present study. Similar results were shown in rice (Mostofa *et al*., 2019) and lettuce (Talabany *et al*., 2023). Study suggested that POD activity enhanced under stress because they can neutralize free radical and eliminate harmful effect of stress (Jaleel *et al*., 2009). A key enzyme in cellular defense known as superoxide dismutase (SOD) stimulates the reduction of superoxide radicals into H_2O_2 and O_2 (Foyer and Noctor, 2000). The application of SA decreased the accumulation of Cd in two melon cultivars (Zhang *et al*., 2015). However activities of antioxidants increase which scavenge Cd-induced excess ROS and maintain an optimum redox homeostasis (Zaid and Mohammad, 2018).

The increase in ROS levels under Cd stress is consistent with the oxidative stress observed in plants. ROS are produced as a byproduct of various metabolic processes, including photosynthesis, and are normally scavenged by the antioxidant system. However, under Cd stress, the increased production of ROS may exceed the antioxidant capacity, leading to oxidative damage. This could contribute to the observed toxicity symptoms in plants. Analysis of this work suggested that salt stress caused a raise in malondialdehyde content. MDA has been suggested to be the primary byproduct of the lipid membrane peroxidation. MDA's composition reflects the cellular membranes' damaged structural state (Parida and Jha, 2010). High-level MDA deposition has been shown to increase O_2 and H_2O_2 under Cd^{+2} toxicity (Xu *et al*., 2014). Under metal stress production of H_2O_2 increased which can damage important cell components. While our observations showed significant changes in the activity of H_2O_2-treated plants. According to a report, lipid peroxidation was brought on by Cd^{+2} stress in the roots of pea seedlings. When hydrogen peroxide increases in concentration then it causes a reduction in photosynthetic pigment and photosystem II activities (Miller *et al*., 2010).

The result showed that the flavonoid content increased under the cadmium stress. These results are similar with Kobyletska *et al*. (2022) in wheat. Flavonoids found in plants have a defensive nature against the harmful effects brought on by various stresses (Alqarawi *et al*., 2014). Flavonoids act as antioxidant compounds that enhance the ability of plants to cope with reactive oxygen species production under stress. Ascorbic acid (AsA) content increased under the application of SA. The results suggested that a higher level of AsA in the varieties might be the reason for its relative more tolerance to stress similar results were observed by (Hassanein *et al*., 2009) in maize. Ascorbic acid have a role in stimulation of growth by decline the harmful effect of salinity on the metabolism of plant and increasing division and enlargement of cells.

In our study, under cadmium stress amino acids and total soluble proteins content increased. Similar results have been shown in rice (Yang *et al*., 2021) and mungbean (Khan *et al*., 2021). Due to stress the soluble proteins and starch content inside the plant cells are degraded with consequent increase soluble proteins. It is supposed under stress other compatible solutes have role in osmotic adjustment help plant to increase storage of reserves to improve plant metabolism under stress (Ismaiel *et al*., 2018). The ability of SA to regulate relies on its concentration, and it is essential for increasing plant tolerance to metal stress by causing the synthesis of amino acid and soluble protein (Sharma *et al*., 2020).

Nutrient uptake is another critical aspect affected by Cd toxicity. The observed decrease in the uptake of essential nutrients, such as nitrogen, phosphorus, and potassium, indicates that Cd may interfere with the nutrient transporters or alter the nutrient demand of the plant (Nazar *et al*., 2012). This could lead to nutrient deficiencies and further compromise plant health. Results of current study reveal that micronutrients such as shoot and root (N, P, and, K) decrease under cadmium stress. Cd limits the uptake of these vital mineral elements (Zaid and Mohammad, 2018). Because excess Cd^{2+} ions have antagonistic effects on ion uptake, Cd contamination in the current study enhanced the endogenous Cd level that inhibits mineral uptake (Nazar *et al*., 2012). Our research supports the conclusions made by Ahmad *et al*. (2011). Nutrient concentrations enhanced in the SA treatment for bean plants subjected to Cd stress. In conclusion, the results of this study provide a comprehensive overview of the effects of Cd stress on plant physiology. The observed impacts on photosynthesis, nutrient uptake, antioxidant systems, ROS levels, and morphological parameters highlight the complexity of Cd toxicity and the need for a holistic approach to mitigate its effects. Future research should focus on understanding the molecular mechanisms underlying these effects and developing strategies to enhance plant resilience against Cd stress.

CONCLUSIONS

Cadmium stress significantly impaired rapeseed growth, reducing photosynthetic pigment concentrations by up to 40%, primarily due to excessive ROS generation and weakened antioxidant defenses. Among the treatments, foliar application of 1 mM SA had the most pronounced effect, leading to a 32% increase in chlorophyll content, a 45% rise in proline levels, and a 38% enhancement in SOD activity compared to Cd-stressed plants without SA. These improvements contributed to reduced oxidative damage, better membrane integrity, and enhanced overall stress tolerance.

These findings underscore the potential of SA as a cost-effective and eco-friendly agrochemical for mitigating heavy metal toxicity in crops. Its ability to strengthen physiological and biochemical defense systems makes it a promising tool in sustainable agricultural practices, especially in metal-contaminated regions. Future studies should incorporate transcriptomic and metabolomic analyses to uncover the molecular basis of SA-induced stress tolerance and conduct field trials to validate its efficacy under real-world farming conditions.

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