Original Research

Advances in Plant Science and Environment

Melatonin Inhibited Cadmium Accumulation and Protected Plant Photosynthetic Machinery in Eggplant Seedlings

Abubakar Abdullahi Lema, Abdulqadir Tahir Ahmad, Faisal Lawal Canada, Murtala Yusuf, Aminu Rabiu Tela, Muhammad Yusuf Abdulkadir, Kabir Usman Kusada, Harisu Yunusa Umar, Hajara Haruna, and Sauban Musa Jibril*

https://doi.org/xx.xxxxx/xxxxx

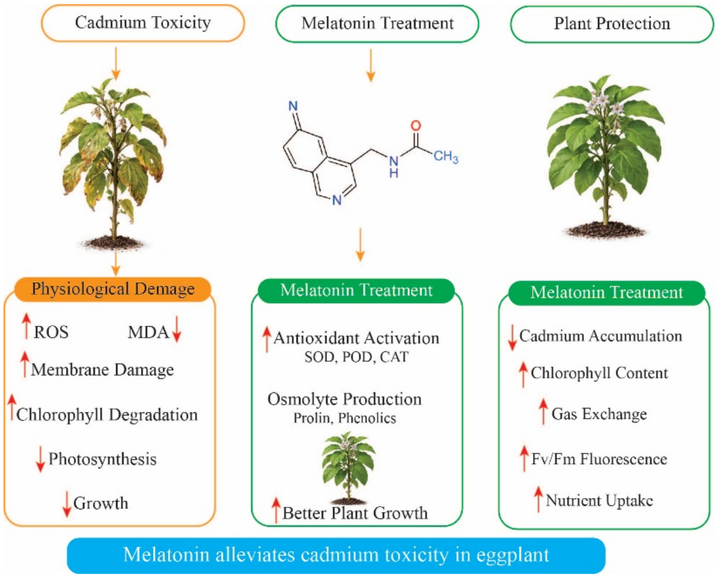
Highlights

- Cd toxicity inhibits eggplant seedling growth and development.
- Cd hindered leaf photosynthetic efficiency and impaired secondary metabolites production.
- MEL treatment positively regulates eggplant growth and antioxidant defense mechanism.

Cite this article as: Lema AA., Ahmad AT., et al., 2024. Melatonin inhibited cadmium accumula tion and protected plant Photosynthetic machinery in eggplant seedlings. Advances in Plant Science and Environment 1, 35-41. https://doi.org/ XXXX X/XXXXX.

Article Info.
Received: 23 June 2024
Accepted: 12 July 2024

Graphical Abstract



Abstract

Cadmium (Cd) is one of the most injurious heavy metals, affecting plant growth and development. Melatonin (MEL), as a plant growth regulator, has been used to alleviate Cd toxicity in many plant species; however, the underlying molecular mechanisms responsible for Cd toxicity in eggplant are still poorly understood. Our results showed that MEL (100 mM), led to positive effects on Cd tolerance, including a significant increase in growth, photosynthetic pigments, and proline content. Exogenous MEL could improve photosynthetic assimilation rate in Cd-treated plants, probably through an increase in proline. Further, MEL led to a decrease in Cd translocation to the shoots. Based on the results, MEL considerably increased antioxidant enzymes (SOD, POD, CAT, APX, GR, GST) activities as well as the production of anthocyanin, total phenols, and flavonoids. The increased activity of antioxidant enzymes led to a decrease in electrolyte leakage (EL), malonaldehyde (MDA), superoxide ion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2) content in the plants exposed to Cd stress. Furthermore, MEL application efficiently increased the mineral nutrient accumulation in plants. In conclusion, the results showed that the use of MEL could reduce oxidative stress and improve biomass in the plants exposed to Cd. These results may be used to develop novel techniques for enhancing agricultural yield sustainably, especially in metal-contaminated soils.

Keywords: Melatonin, cadmium, photosynthesis, secondary metabolites, antioxidant enzymes

1. Introduction

Heavy metal pollution has become one of the most serious environmental problems worldwide with the development of industries, agriculture, and urbanization, including China (Li et al., 2022). Cadmium (Cd), a toxic element of heavy metal pollutants, is widely distributed in the soil, and its accumulation in plants threatens human health through the food chains (Wang et al., 2021). Excess Cd accumulation in plants can induce excessive reactive oxygen species (ROS) generation, resulting in an imbalance between ROS generation and scavenging. This would cause lipid peroxidation and oxidative damage, destroy cell membranes, and disturb the function of chlorophyll, consequently, eventually reducing crop yields (Gallego et al., 2012; Haider et al., 2021). Therefore, it is imperative to reduce Cd accumulation and minimize the entry of Cd into the food chain. Plants have evolved complex biochemical and molecular mechanisms for detoxification of Cd toxicity, including the transportation, sequestration, detoxification of Cd ions, and the synthesis of antioxidants, signaling molecules, stress-related proteins, and hormones (Hasan et al., 2015; Tousi et al., 2020; Xu et al., 2020). It has been reported that plant hormones, including MEL, salicylic acid, strigolactone, and jasmonic acid, play crucial roles in response to Cd toxicity by regulating the synthesis of antioxidants, signaling molecules, and others in different plant species (Kaya et al., 2019; Imran et al., 2021; Shah et al., 2023).

Melatonin (MEL) is a well-known master plant growth regulator and has antioxidant properties. It regulates physiological roles, including inhibition of seedling growth, modified root growth pattern, increased metabolites production, protected photosynthetic apparatus, balanced mineral homeostasis, and improved tolerance to heavy metal stress of plants (Yang et al., 2023; Altaf et al., 2023). Several reported suggested that MEL increases the plant tolerance to various abiotic stresses, such as salinity (Zhang et al., 2024), drought (Liu et al., 2015), low and high temperature (Jahan et al., 2019; Qari et al., 2022), UV-radiation (Wei et al., 2019), and heavy metals (Parwez et al., 2023; Rizwan et al., 2024). MEL induces Cd stress tolerance in *Brassica napus* L., *Solanum nigrum* L., and *Cucumis sativus* L. by increasing the strength of the antioxidant defense system and reducing the Cd content which was attributed to Cd stress tolerance (Shah et al., 2020; Teng et al., 2022). In this way, MEL may play a critical function in reducing stress-induced damage. Another report showed an active role of MEL to enhance secondary metabolites accumulation and maintain mineral homeostasis in tomato under Cd cadmium toxicity (Altaf et al., 2022a). Foliar application of MEL in leaves of tobacco enhanced Cd tolerance by promoting cell wall and vacuolar sequestration of Cd, and regulating the expression of Cd-related genes in roots (Wang et al., 2019). Hasan et al. (2015) reported that MEL application remarkably improved seedling growth, photosynthetic system and antioxidant enzyme system and inhibited Cd accumulation from root to shoot in tomato seedlings. Wang et al. (2021) reported that exogenously applied MEL alleviated Cd stress in Chinese cabbage seedlings by inhibiting Cd accumulation.

The major constituent of the human diet is vegetables, which are believed to be a vital source of important vitamins, nutrients, fibers, metabolites, and antioxidants (Zhou et al., 2023). Among these vegetables, eggplant (*Solanum melongena* L.) belong to the family Solanaceae, is produced all over the world (Kaur and Das, 2023). It is commonly known as brinjal and is among the most widely consumed vegetables in tropical and sub-tropical areas. The present study focused on the effect of MEL application, used as a foliar application, on redox homeostasis, photosynthesis, antioxidant enzymes, and growth of eggplant under Cd stress. Consequently, the objective of the research study was to enhance the eggplant's capacity to endure Cd stress with the least number of adverse impacts.

2. Materials and Methods

2.1. Experimental Setup

The seeds of the eggplant cultivar Purple Shine were sown in seedling trays filled with nutrient-rich soil. After 25 days of sowing, the transplantation of eggplant seedlings was done at three true leaves stage into black plastic pots filled with nutrient-rich soil. The seedlings were pre-cultivated for seven days to allow the adaptation to new conditions. Then, concentrations of MEL (100 mM) were applied directly into the soil for ten days. After MEL pretreatment, seedlings were irrigated with 50 µM $CdCl_2$. Control plants were irrigated with distilled water without the addition of Cd or MEL. After seven days of Cd treatment, Quick freezing of all leaf samples was done by adding liquid nitrogen and storing the samples at a low temperature of -80 OC. The treatments were as follows: (1) CK (control); (2) MEL (melatonin); (3) Cd (cadmium); (4) MEL + Cd (melatonin + cadmium). The whole experiment was conducted under controlled condition.

2.2. Determination of Plant Biomass, Mineral and Cd Content

The biomass of eggplant plants was assessed by determining the fresh and dry weight. Electric balance was used to measure the fresh weight of the plant. Plants were oven-dried (80 °C for 72 h) for dry weight records. Cd contents in the oven-dried eggplant roots and leaves were quantified following the procedure described by Altaf et al. (2023). Cao et al. (2019) previously reported the measurement of mineral nutrient (Nitrogen, Phosphorus, and potassium) content in eggplant leaves and roots.

2.3. Determination of Gas Exchange Parameters

After seven days of Cd treatments, the net photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration rate (Tr) of the fully expanded leaf were determined using an infrared gas analyzer portable photosynthesis system (Nawaz et al., 2018).

2.4. Measurement of photosynthetic pigments

100 mg of leaf samples from each set was chopped and extracted with 80% acetone. The levels of chlorophyll a, chlorophyll b, and total Chlorophyll, and carotenoids in the freshly harvested leaves were spectrophotometrically quantified according to the formula reported by Lichtenthaler and Wellburn (1983).

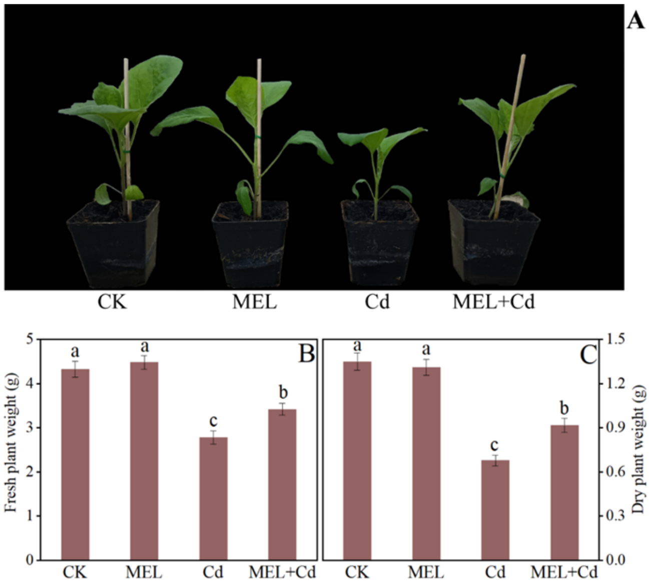


Fig. 1. Influence of melatonin on plant growth performance and growth traits in eggplant seedlings under cadmium toxicity. Means ± SE, n = 3, lowercase letter showed a significant difference among treatments, according to Fisher LSD test.

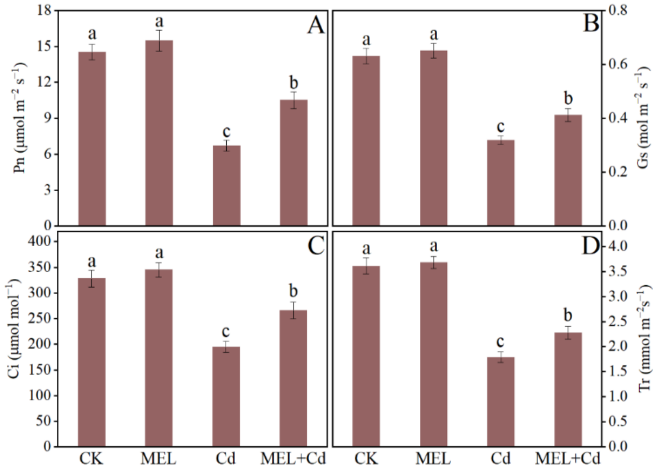


Fig. 2. Influence of melatonin on leaf gas exchange parameters in eggplant seedlings under cadmium toxicity. Means ± SE, n = 3, lowercase letter showed a significant difference among treatments, according to Fisher LSD test.

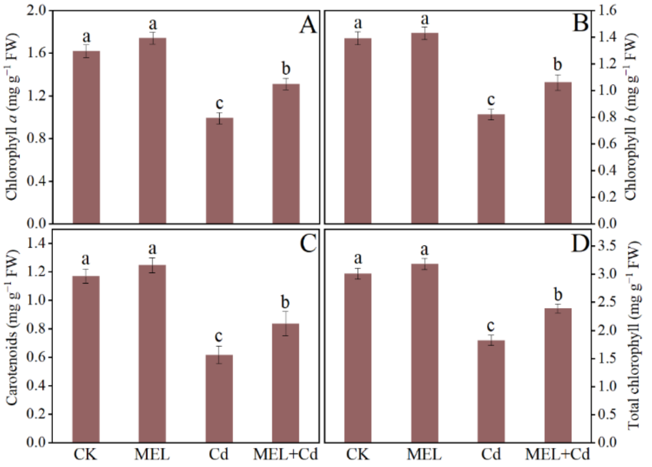


Fig. 3. Influence of melatonin on photosynthetic pigment content in eggplant seedlings under cadmium toxicity. Means ± SE, n = 3, lowercase letter showed a significant difference among treatments, according to Fisher LSD test.

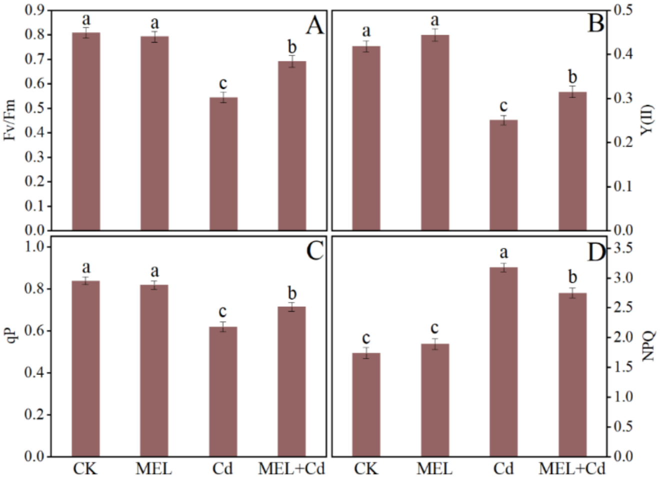


Fig. 4. Influence of melatonin on chlorophyll fluorescence characteristics in eggplant seedlings under cadmium toxicity. Means ± SE, n = 3, lowercase letter showed a significant difference among treatments, according to Fisher LSD test.

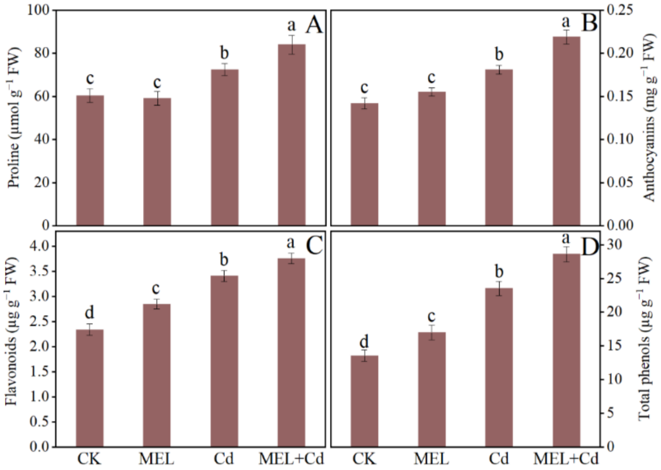


Fig. 5. Influence of melatonin on proline and secondary metabolites production in eggplant seedlings under cadmium toxicity. Means ± SE, n = 3, lowercase letter showed a significant difference among treatments, according to Fisher LSD test.

2.5. Chlorophyll Fluorescence Parameters

To measure chlorophyll fluorescence (CF), fully formed leaves were utilized, and leaf data were obtained between 9:00 and 11:00 a.m. using an IMAGING-PAM Chlorophyll fluorescence analyzer (Heinz Walz, Effeltrich, Germany) after 30 min of dark adaptation. The Fv/Fm (maximum photochemical efficiency) value was determined in accordance with (Altaf et al., 2022b).

2.6. Proline and Secondary Metabolites Analysis

Bates et al. (1973) described a method for determining the proline content. A 0.5 g leaf sample was homogenized in 5 mL sulfosalicylic acid (3% m/v) and centrifuged at 12,000 g for 20 min at 4 °C. The content of the proline was measured at 520 nm. Leaf samples (0.2 g) were crushed using cold methanol (70% v/v) having formic acid (2% v/v) and ethanol (28% v/v), for measuring the secondary metabolites. Anthocyanin, flavonoids, and total phenols were measured according to the procedure quoted by Jahan et al., (2020).

2.7. Oxidative Stress Biomarkers and Antioxidant Enzymes Analysis

The ground tissue (leaf) samples were homogenized in 900 mL of 100 mM phosphate buffer (pH 7.4), as prescribed in the kit. Each homogenized sample was centrifuged at 12,000_ g for 15 min at 4 °C. After that, the supernatant was added to a new Falcon tube for further analysis. SOD, CAT, APX, GR, POD, and GST were measured according to the method quoted by (Nawaz et al., 2018; Yin et al., 2019; Jahan et al., 2020). H_2O_2 and $O_2^{\bullet-}$ were determined using the method proposed by Ibrahim and Jaafar (2012); Zhang et al. (2019). Integrity of membrane and lipid peroxidation was detected by measuring the MDA levels through the TBA (thiobarbituric acid) reaction (Wang et al., 2010). The EL (electrolyte leakage) ratio was estimated in the previously reported procedure (Gong et al., 1998).

2.8. Statistical Analysis

The statistical analysis was carried out using the Statistics 10.1 software. Different letters indicate a significant difference among treatments ($p \leq 0.05$). The values in the figures are always expressed as the mean standard error of four independent replicates. Fisher's least significant difference (LSD) ($p \leq 0.05$) test was used to determine the differences among treatments.

3. Results

3.1. Plant Growth

Cd treatment showed a significant decline in the growth of eggplant seedlings compared with CK treatment (Fig. 1A). The eggplant seedlings, when exposed to Cd toxicity, significantly reduced in plant fresh weight (35.97%) and plant dry weight (49.85%), compared with CK plants (Fig. 1B-C). This decline in the growth of eggplant seedlings was alleviated by the exogenous application of MEL (Fig. 1A-C). After pretreatment with MEL, growth limitations caused by Cd stress were improved, and less reduction in plant fresh weight (32.07%) and plant dry weight (21.06%) was observed (Fig. 1). Further, no difference was seen in seedlings without ME pretreatment (control) and seedlings treated with 100 mM MEL under no stress conditions.

3.2. Photosynthesis Related Parameters

Under Cd stress, Pn, Gs, Ci, and Tr were decreased by 53.18%, 49.44%, 44.54%, and 50.41%, respectively, compared with CK seedlings (Fig. 2A-D). Conversely, when seedlings were treated with MEL, the reductions of these leaf gas exchange parameters were only 27.66%, 34.71%, 19.01%, and 36.84%, respectively, as compared to the CK plants (Fig. 2A-D).

A similar trend of reduction in the pigment system was observed under the Cd stress condition. Chlorophyll a, chlorophyll b, carotenoid, and total chlorophyll content in the leaves of eggplants were reported to be sharply reduced by 38.88%, 41.01%, 47.26%, and 39.53%, respectively, under Cd stress (Fig. 3A-D). Per contra, MEL-treated eggplant seedlings, when subjected to Cd treatment, these pigments' content significantly increased—by 32.32%, 29.26%, 35.65%, and 31.65%, respectively—when compared with only Cd-stressed seedlings (Fig. 3A-D).

As shown in Fig. 4A-D, Cd treatment considerably decreased the Fv/Fm, PSII (Y(II)), and qP, and increased the NPQ value in eggplant seedlings. On the other hand, the values of Fv/Fm, PSII (Y (II)), and qP increased while the values of NPQ reduced with MEL application in eggplant seedlings exposed to Cd stress (Fig. 4A–D).

3.3. Proline And Secondary Metabolites

Proline, anthocyanin, flavonoids, and total phenolic content were remarkably increased (19.95%, 27.46%, 45.72% and 73.70%, respectively) under the Cd stress compared with CK plants. Per contra, MEL treatment further increased the proline content (15.98%), anthocyanin (20.99%), flavonoids (10.26%), and total phenolic content (21.68%) compared with Cd-stressed seedlings (Fig. 5A-D).

3.4. Oxidative Stress Biomarkers

After seven days of Cd treatment, the H_2O_2, $O_2^{\bullet -}$, MDA, and EL levels were measured in the leaves of eggplant seedlings (Fig. 6). For instance, in the eggplant seedlings under the Cd toxicity, the H_2O_2, $O_2^{\bullet -}$, MDA, and EL levels significantly increased by 2.11-, 0.86-, 0.68-, and 1.96-fold, respectively, compared with CK seedlings. Importantly, MEL-pretreated plants subjected to Cd-stress reduced this content only by 0.27-, 0.12-, 0.22-, and 0.21-fold, respectively, compared with the Cd treatment (Fig. 6).

3.5. Antioxidant Enzyme Activity

The antioxidant enzymes' (SOD, CAT, POD, APX, GST, and GR) activities were measured in the leaves of eggplant (Fig. 7). By exposure to Cd stress, the SOD, CAT, POD, APX, GST, and GR enzymes activity was enhanced by 66.32%, 62.85%, 23.44%, 68.01%-, 38.07%%, and 28.57%, respectively compared to CK seedlings. It is noteworthy that when MEL-treated seedlings were subjected to Cd-treatment, this further elevated these antioxidant enzymes by 34.29%, 44.56%, 19.19%, 38.15%, 23.16%, and 28.35%, respectively, compared with the Cd-stress group (Fig. 7A–F).

3.6. Ion Analysis

The mineral nutrient contents of roots and leaves are determined for elucidating the role of MEL applied exogenously in nutrients' homeostasis under Cd stress conditions, and the findings showed a decline in mineral nutrient contents of roots and leaves by Cd-stress in pepper (Fig. 8). The reduction in N, P, and K content can be seen in both leaves and roots of Cd-treated seedlings. Contrarily, the detrimental effects of Cd stress were positively retrieved by the exogenous supplementation of MEL, which remarkably increases the N, P, and K concentrations of leaves and roots, thus highlighting the role of MEL in eggplant as a key player in sustaining the mineral nutrients pool (Fig. 8). To elucidate the alleviating effect of Cd through inhibition of Cd uptake under Cd-stress conditions, the Cd accumulation of metal-stressed plants in both roots and leaves was estimated. The results revealed a tremendous increase in Cd concentration in both roots and leaves of Cd -treated seedlings plants, and the leaves showed substantially higher uptake level of Cd than the roots (Fig. 9). Nevertheless, compared to Cd-stressed plants only, the concentrations of Cd in both roots and leaves of pepper plants were markedly declined by the exogenous supplementation of MEL.

4. Discussion

Global agricultural environmental research is primarily focused on reducing heavy metal contamination in agricultural soils and heavy metal toxicity in agricultural soils, both of which are serious problems. Cd is a non-essential and highly toxic metal that reduces plant growth (Gallego et al., 2012). According to recent research, MEL may effectively enhance heavy metal stress tolerance in crops, providing a novel approach for reducing heavy metal toxicity in crops (Zhou et al., 2023; Yang et al., 2023). In this study, we observed that Cd impaired the plant growth traits. Conversely, Cd-induced growth inhibition was progressively limited by MEL pre-treatment (Fig. 1). In the last decade, several researchers have been concerned about MEL role in boosting plant growth and stress tolerance (Kaya et al., 2019; Tousi et al., 2020; Hoque et al., 2021). Under metal toxicity in wheat and tomato, growth traits' improvement mediated by MEL is in line with existing literature (Li et al., 2022; Umapathi et al., 2022). Under nickel toxicity, MEL application promoted Trigonella foenum-graecum seedling growth (Parwez et al., 2023). As a result, exogenous MEL supplementation may be a highly promising approach for dealing with the negative impacts of heavy metal toxicity on plants.

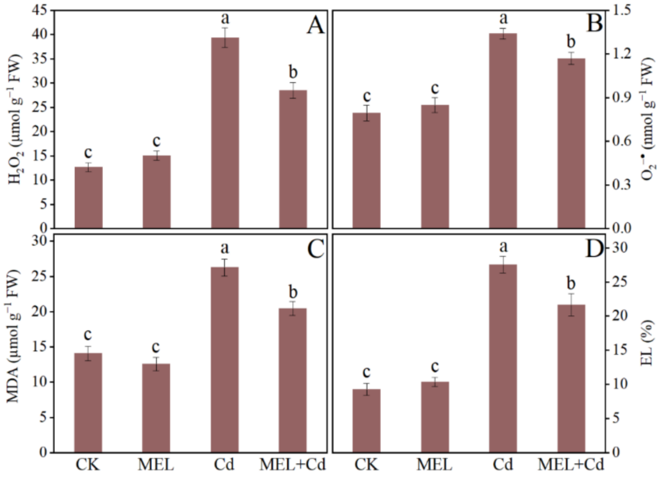


Fig. 6. Influence of melatonin on oxidative stress biomarkers in eggplant seedlings under cadmium toxicity. Means ± SE, n = 3, lowercase letter showed a significant difference among treatments, according to Fisher LSD test.

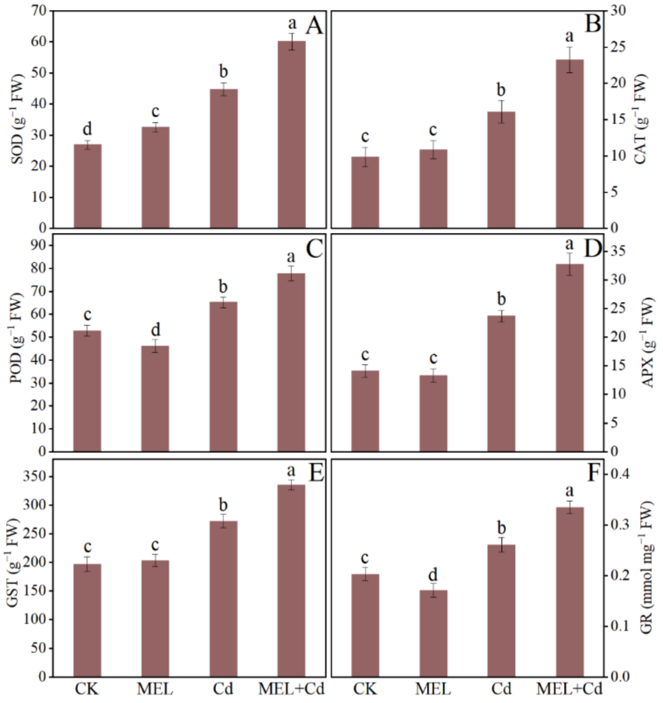


Fig. 7. Influence of melatonin on antioxidant enzyme activity in eggplant seedlings under cadmium toxicity. Means ± SE, n = 3, lowercase letter showed a significant difference among treatments, according to Fisher LSD test.

Heavy metal toxicity impairs leaf photosynthesis (Altaf et al., 2023). The capacity of plants to survive negative environmental conditions is associated with the ability to enhance their photosynthetic capacity. Inhibition of photosynthesis by heavy metals leads to the impairment of the photosynthetic apparatus (Yang et al., 2023). Increased Cd ion concentration in leaf tissue might be the primary source of chloroplast and leaf function inhibition in cotton plants subjected to Cd toxicity (Khan et al., 2023). The current results exhibited that only Cd treatment dramatically reduced leaf gas exchange and photosynthetic pigments content. In contrast, MEL supplementation clearly activated the photosynthesis mechanism (Fig. 2 and Fig. 3). Similarly, Nawaz et al. (2018) reported that V received watermelon seedlings showed considerable reduction in leaf gas exchange parameters. But the MEL application effectively retrieved the value of these parameters under vanadium toxicity. Further, Ahammed et al. (2020) revealed that iron supply, at both low and high levels, caused a considerable reduction in chlorophyll a and chlorophyll b content compared with CK. Interestingly, cucumber seedling pretreatment with MEL conspicuously improved the chlorophyll a and chlorophyll b contents under low and high iron supply compared with only iron treatments. Numerous research has shown that exogenous MEL supplementation recovers leaf photosynthesis in Chinese cabbage (Wang et al., 2021), radish (Xu et al., 2020), and *Brassica napus* L. (Sami et al., 2020) under Cd toxicity. Chlorophyll fluorescence has become an important technique for studying the photosynthetic parameters of plants under adverse environmental conditions (Jahan et al., 2021). The present study revealed that Cd treatment caused significant damage to the photosynthetic apparatus in eggplant seedlings, which was then recovered by MEL supplementation (Fig. 4). The current study also confirms the results of previous studies, which showed that MEL might protect photosynthetic efficiency (Saqib et al., 2023). Furthermore, the results of the current study are also in line with the existing literature on strawberry (Wu et al., 2021), Spinach (Asif et al., 2020), and mallow (Tousi et al., 2020).

Under stressed conditions, proline, a principal metabolite, is produced in the cells of plants. The research findings of present study revealed that Cd treatment enhanced the proline concentration, whereas application of MEL in seedlings of eggplant under Cd stress further enhanced the proline level (Fig. 5A). Identically, application of MT increased the proline in tomato, pepper and eggplant leaves (Jahan et al., 2020; Altaf et al., 2023; Zhang et al., 2024) under environmental stress. In addition, anthocyanins, flavonoids, and total phenolics have great potential to protect plants against stress (Rizwan et al., 2024). In comparison to other treatments, MT application enhanced the total phenolics, flavonoids, and anthocyanins in seedlings of eggplant under Cd stress (Fig. 5D-F). Our research findings are further confirmed by findings that the application of MT in tomato has the same impact under nickel toxicity (Jahan et al., 2020). Farouk and Al-Amri (2019) indicate that higher production of anthocyanin can increase antioxidant capacity and decrease H_2O_2 level. Similarly, Zhang et al. (2016) reported that ME supplementation sharply enhanced anthocyanin concentration in cabbage and therefore showed decreased ROS accumulation as well as an increased antioxidant enzyme system.

Plants can be protected from oxidative stress by MEL, which directly enhances the activity of antioxidative enzymes and scavenges free radicals and excess ROS (Hoque et al., 2021). Our results show that the elevations of EL and MDA were consistent with the accumulation of H_2O_2 and $O_2^{\bullet -}$ induced by Cd stress, which indicates possible Cd-induced damage of the membrane due to excess ROS (Fig. 6). In contrast, MEL application significantly reduced these oxidative stress biomarkers. These results are in accordance with previous studies that MEL application decreased the accumulation of ROS and ROS-induced lipid peroxidation in wheat (Li et al., 2022), tobacco (Teng et al., 2022), and Chinese cabbage (Wang et al., 2021) under Cd toxicity. In addition, MEL application considerably decreased the H_2O_2 and MDA accumulation in watermelon seedlings under vanadium toxicity (Nawaz et al., 2018). Additionally, our results are in line with several previous studies on wheat, mallow, and seedlings that were exposed to heavy metal toxicity (Tousi et al. 2020; Imran et al., 2021; Li et al., 2022).

Redox homeostasis and are crucial in limiting excessive production of ROS and the duration of cellular membrane damage in plants under environmental stresses (Qin et al., 2024). MEL is a dynamic molecule having antioxidant properties (Chrustek et al., 2021). MEL effectively up-regulated antioxidant enzyme activity and decreased oxidative damage in plants (Fig. 7). In tobacco plants, MEL efficiently improved the antioxidant activity by decreasing ROS production, primarily related to enhancing tobacco manganese tolerance (Gao et al., 2022). Jahan et al. (2020) reported that under Ni stress, MEL application increased antioxidant enzyme activity in the roots and leaves of tomato seedlings. MEL application notably enhanced enzyme activity in safflower (Amjadi et al. 2021), pearl millet (Awan et al. 2023), and Spinach (Asif et al., 2020) under cadmium toxicity.

The current study exhibited greater Cd accumulation in Cd-stressed seedlings, whereas less Cd was absorbed by MEL pretreated seedlings, with a relatively higher Cd concentration in roots than in leaves (Fig. 8). Excessive Cd interferes nutrients uptake and inhibits root growth (Saqib et al., 2023). Similarly, MEL application considerably decreased Cd accumulation in strawberry seedlings (Wu et al., 2021) and tomato (Song et al., 2024). An accentuated decline in N, P, and K was observed under Cd toxicity, whereas robust improvements were observed in these nutrient elements by MEL supplementation under Cd stress, thus implying MEL as an essential ion homeostasis booster in plants (Fig. 9). Similar results were reported in cucumber under copper toxicity (Cao et al., 2019). These research results are in agreement with previous findings that the application of MEL enhanced mineral homeostasis (NPK) in watermelon (Nawaz et al., 2018), wheat (Li et

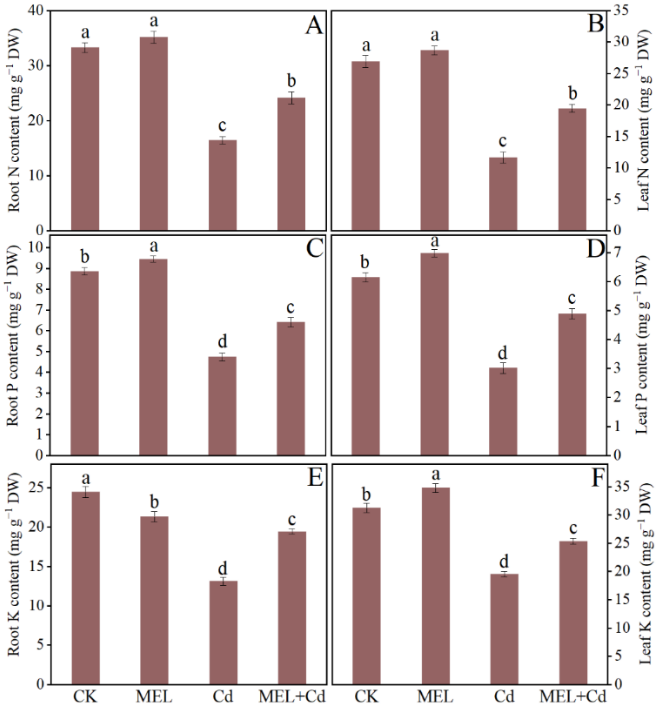


Fig. 8. Influence of melatonin on mineral nutrient accumulation in eggplant seedlings under cadmium toxicity. Means ± SE, n = 3, lowercase letter showed a significant difference among treatments, according to Fisher LSD test.

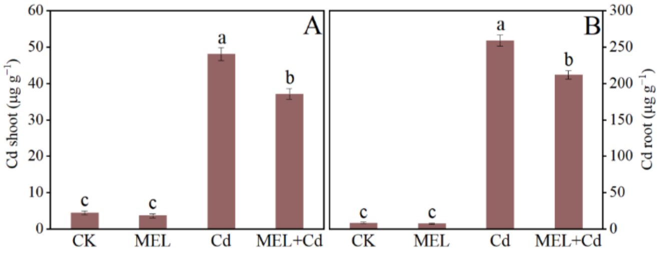


Fig. 9. Influence of melatonin on cadmium accumulation in eggplant seedlings under cadmium toxicity. Means ± SE, n = 3, lowercase letter showed a significant difference among treatments, according to Fisher LSD test.

al., 2022), radish (Xu et al., 2020), and pepper (Rizwan et al., 2024) subjected to heavy metal stress conditions. Our results also endorsed that MEL assisted in enhancing the tolerance against Cd stress through conserving ion homeostasis in seedlings of eggplants. MEL is a powerful antioxidant molecule and significantly increases heavy metals stress tolerance in plant species.

5. Conclusion

The current study exhibited that Cd stress causes significant declines in growth, interruptions in the photosynthetic apparatus, imbalances ion homeostasis, as well as excess accumulation of ROS, which all together reduce the tolerance of eggplant seedlings against Cd-toxicity. Conversely, MEL proficiently mitigated the Cd-induced phytotoxicity by restoring growth attributes, increasing leaf photosynthetic capacity, balancing ion accumulation (decreasing the Cd accumulation in seedlings), and decreasing oxidative damage (hindering the ROS accumulation and increasing antioxidant defense mechanism). Additionally, MEL-treated eggplant seedlings had higher levels of secondary metabolites, which primarily take part in Cd chelation and reduce ROS generation, relieving the Cd-induced growth suppression. To sum up, the MEL application effectively alleviated Cd-induced phytotoxicity and enhanced eggplant seedlings' resilience to Cd toxicity. Given the results of this study, further research is needed to determine the function of MEL in protecting plants against various environmental pollutants such as arsenic, lead, and selenium.

Author Information

Corresponding Author

Sauban Musa Jibril: Department of Biological Sciences, College of Natural and Applied Science, Al-Qalam University, Katsina 2137, Katsina, Nigeria.
Email: saubanzango@gmail.com
ORCID: https://orcid.org/0000-0002-9631-2660

Author(s)

Abubakar Abdullahi Lema: Department of Biological Sciences, College of Natural and Applied Science, Al-Qalam University, Katsina 2137, Katsina, Nigeria.
ORCID: https://orcid.org/0000-0001-9505-9723

Abdulqadir Tahir Ahmad: Department of Biological Sciences, Federal University of Kashere, Gombe State, Nigeria.
ORCID: https://orcid.org/0000-0003-0917-3963

Faisal Lawal Canada: Department of Biochemistry, College of Natural and Applied Science, Al-Qalam University, Katsina 2137, Katsina, Nigeria.
ORCID: https://orcid.org/0009-0004-9706-922X

Murtala Yusuf: Department of Biological Sciences, College of Natural and Applied Science, Al-Qalam University, Katsina 2137, Katsina, Nigeria.

Aminu Rabiu Tela: Department of Biological Sciences, College of Natural and Applied Science, Al-Qalam University, Katsina 2137, Katsina, Nigeria.
ORCID: https://orcid.org/0000-0003-2919-8834

Muhammad Yusuf Abdulkadir: Department of Food Science and Technology, Aliko Dangote University of Science and Technology, Wudil, Nigeria.
ORCID: https://orcid.org/0009-0003-1107-5016

Kabir Usman Kusada: Department of Biological Sciences, Federal University Dutsin-Ma, Katsina State, Nigeria.

Harisu Yunusa Umar: Department of plant Science and Biotechnology, Bayero University Kano, Nigeria.
ORCID: https://orcid.org/0009-0005-5404-5081

Hajara Haruna: Department of plant Science and Biotechnology, Bayero University Kano, Nigeria.

Author Contributions

AAL, conceptualization and writing original draft, reviewing and editing; ATA, formal analysis and reviewing and editing; FLC, reviewing and editing; MY, data validation and reviewing and editing; ART, formal analysis, validation; MYA, formal analysis; KUK, formal analysis; HYU, formal analysis, HH, formal analysis; and SMJ, conceptualization, formal analysis, reviewing and editing, funding, supervision.

Competing Interests

The authors have no relevant financial or non–financial interests to disclose.

Data availability

All data generated or analyzed during this study are included in this article.

Additional Information

References

Ahammed, G.J., Wu, M., et al., 2020. Melatonin alleviates iron stress by improving iron homeostasis, antioxidant defense and secondary metabolism in cucumber. Scientia Horticulturae 265, 109205. https://doi.org/10.1016/j.scienta.2020.109205

Altaf, M.A., Shahid, R., et al., 2022a. Melatonin mitigates cadmium toxicity by promoting root architecture and mineral homeostasis of tomato genotypes. Journal of Soil Science and Plant Nutrition 22,1112-1128. https://doi.org/10.1007/s42729-021-00720-9

Altaf, M.A., Sharma, N., et al., 2023. Deciphering the melatonin-mediated response and signalling in the regulation of heavy metal stress in plants. Planta 257, 115. https://doi.org/10.1007/s00425-023-04146-8

Altaf, M.A., Shu, H., 2022b. Melatonin affects the photosynthetic performance of pepper (*Capsicum annuum* l.) seedlings under cold stress. Antioxidants 11, 2414. https://doi.org/10.3390/antiox11122414

Amjadi, Z., Namdjoyan, S., et al., 2021. Exogenous melatonin and salicylic acid alleviate cadmium toxicity in safflower (*Carthamus tinctorius* L.) seedlings. Ecotoxicology 30, 387-401. https://doi.org/10.1007/s10646-021-02364-y

Asif, M., Pervez, A., et al., 2020. Melatonin and plant growth-promoting rhizobacteria alleviate the cadmium and arsenic stresses and increase the growth of *Spinacia oleracea* L. Plant, Soil and Environment 66, 234-241. https://doi.org/10.17221/135/2020-pse

Awan, S.A., Khan, I., et al., 2023. Reduction in the cadmium (Cd) accumulation and toxicity in pearl millet (*Pennisetum glaucum* L.) by regulating physio-biochemical and antioxidant defense system via soil and foliar application of melatonin. Environmental Pollution 328, 121658. https://doi.org/10.1016/j.envpol.2023.121658

Bates, L.S., Waldren, R.A., 1973. Rapid determination of free proline for water-stress studies. Plant and soil 39, 205-207. https://doi.org/10.1007/bf00018060

Cao, Y.Y., Qi, C.D., et al., 2019. Melatonin alleviates copper toxicity via improving copper sequestration and ROS scavenging in cucumber. Plant and Cell Physiology 60, 562-574. https://doi.org/10.1093/pcp/pcy226

Chrustek, A., and Olszewska-Słonina, D., 2021. Melatonin as a powerful antioxidant. Acta Pharmaceutica 71, 335-354. https://doi.org/10.2478/acph-2021-0027

Farouk, S., and Al-Amri, S.M., 2019. Exogenous melatonin-mediated modulation of arsenic tolerance with improved accretion of secondary metabolite production, activating antioxidant capacity and improved chloroplast ultrastructure in rosemary herb. Ecotoxicology and Environmental Safety 180, 333-347. https://doi.org/10.1016/j.ecoenv.2019.05.021

Gallego, S.M., Pena, L.B., et al., 2012. Unravelling cadmium toxicity and tolerance in plants: insight into regulatory mechanisms. Environmental and Experimental Botany 83, 33-46. https://doi.org/10.1016/j.envexpbot.2012.04.006

Gao, H., Huang, L., et al., 2022. Exogenous melatonin application improves resistance to high manganese stress through regulating reactive oxygen species scavenging and ion homeostasis in tobacco. Plant Growth Regulation 98, 219-233. https://doi.org/10.1007/s10725-022-00857-2

Gong, M., Li, Y.J., et al., 1998. Abscisic acid-induced thermotolerance in maize seedlings is mediated by calcium and associated with antioxidant systems. Journal of Plant Physiology 153, 488-496. https://doi.org/10.1016/s0176-1617(98)80179-x

Haider, F.U., Liqun, C., et al., 2021. Cadmium toxicity in plants: Impacts and remediation strategies. Ecotoxicology and Environmental Safety 211, 111887. https://doi.org/10.1016/j.ecoenv.2020.111887

Hasan, M.K., Ahammed, G.J., et al., 2015. Melatonin mitigates cadmium phytotoxicity through modulation of phytochelatins biosynthesis, vacuolar sequestration, and antioxidant potential in *Solanum lycopersicum* L. Frontiers in Plant Science, 6, 601. https://doi.org/10.3389/fpls.2015.00601

Hoque, M.N., Tahjib-Ul-Arif, M., et al., 2021. Melatonin modulates plant tolerance to heavy metal stress: Morphological responses to molecular mechanisms. International Journal of Molecular Sciences 22, 11445. https://doi.org/10.3390/ijms222111445

Ibrahim, M.H., and Jaafar, H.Z., 2012. Primary, secondary metabolites, H_2O_2, malondialdehyde and photosynthetic responses of Orthosiphon stimaneus Benth. to different irradiance levels. Molecules, 17, 1159-1176. https://doi.org/10.3390/molecules17021159

Imran, K.H.A.N., Seleiman, M.F., et al., 2021. Enhancing antioxidant defense system of mung bean with a salicylic acid exogenous application to mitigate cadmium toxicity. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 49, 12303-12303. https://doi.org/10.15835/nbha49212303

Jahan, M.S., Guo, S., et al., 2020. Melatonin alleviates nickel phytotoxicity by improving photosynthesis, secondary metabolism and oxidative stress tolerance in tomato seedlings. Ecotoxicology and Environmental Safety 197, 110593. https://doi.org/10.1016/j.ecoenv.2020.110593

Jahan, M.S., Guo, S., et al., 2021. Melatonin-mediated photosynthetic performance of tomato seedlings under high-temperature stress. Plant Physiology and Biochemistry 167, 309-320. https://doi.org/10.1016/j.plaphy.2021.08.002

Jahan, M.S., Shu, S., et al., 2019. Melatonin alleviates heat-induced damage of tomato seedlings by balancing redox homeostasis and modulating polyamine and nitric oxide biosynthesis. BMC Plant Biology 19, 414. https://doi.org/10.1186/s12870-019-1992-7

Kaur, Y. ,and Das, N., 2023. Roles of polyamines in growth and development of the Solanaceous crops under normal and stressful conditions. Journal of Plant Growth Regulation 42, 4989-5010. https://doi.org/10.1007/s00344-022-10841-9

Kaya, C., Okant, M., et al., 2019. Melatonin-mediated nitric oxide improves tolerance to cadmium toxicity by reducing oxidative stress in wheat plants. Chemosphere 225, 627-638. https://doi.org/10.1016/j.chemosphere.2019.03.026

Khan, A., Jie, Z., et al., 2023. Pretreatment of melatonin rescues cotton seedlings from cadmium toxicity by regulating key physio-biochemical and molecular pathways. Journal of Hazardous Materials 445, 130530. https://doi.org/10.1016/j.jhazmat.2022.130530

Li, G.Z., Wang, Y.Y., et al., 2022. Exogenous melatonin mitigates cadmium toxicity through ascorbic acid and glutathione pathway in wheat. Ecotoxicology and Environmental Safety 237, 113533. https://doi.org/10.1016/j.ecoenv.2022.113533

Lichtenthaler, H.K., and Wellburn, A.R., 1983. Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochemistry Society Transaction 11, 591–592. https://doi.org/10.1042/bst0110591

Liu, J., Wang, W., et al., 2015. Exogenous melatonin improves seedling health index and drought tolerance in tomato. Plant Growth Regulation 77, 317-326. https://doi.org/10.1007/s10725-015-0066-6

Nawaz, M.A., Jiao, Y., et al., 2018. Melatonin pretreatment improves vanadium stress tolerance of watermelon seedlings by reducing vanadium concentration in the leaves and regulating melatonin biosynthesis and antioxidant-related gene expression. Journal of Plant Physiology 220, 115-127. https://doi.org/10.1016/j.jplph.2017.11.003

Parwez, R., Aqeel, U., et al., 2023. Melatonin supplementation combats nickel-induced phytotoxicity in *Trigonella foenum*-graecum L. plants through metal accumulation reduction, upregulation of NO generation, antioxidant defence machinery and secondary metabolites. Plant Physiology and Biochemistry 202, 107981. https://doi.org/10.1016/j.plaphy.2023.107981

Qari, S.H., Hassan, M.U., et al., 2022. Melatonin induced cold tolerance in plants: physiological and molecular responses. Frontiers in Plant Science 13, 843071. https://doi.org/10.3389/fpls.2022.843071

Qin, C., Lian, H., et al., 2024. Chromium mediated damaging effects on growth, nitrogen metabolism and chlorophyll synthesis in tomato can be alleviated by foliar application of melatonin and jasmonic acid priming. Scientia Horticulturae 323, 112494. https://doi.org/10.1016/j.scienta.2023.112494

Rizwan, M., Nawaz, A., et al., 2024. Exogenously applied melatonin enhanced chromium tolerance in pepper by up-regulating the photosynthetic apparatus and antioxidant machinery. Scientia Horticulturae 323, 112468. https://doi.org/10.1016/j.scienta.2023.112468

Sami, A., Shah, F.A., et al., 2020. Melatonin mitigates cadmium and aluminium toxicity through modulation of antioxidant potential in *Brassica napus* L. Plant Biology 22, 679-690. https://doi.org/10.1111/plb.13093

Saqib, M., Shahzad, U., et al., 2023. Exogenous melatonin alleviates cadmium-induced inhibition of growth and photosynthesis through upregulating antioxidant defense system in strawberry. South African Journal of Botany 157, 10-18. https://doi.org/10.1016/j.sajb.2023.03.039

Shah, AA., Ahmed, S., et al., 2020. Cadmium stress consolation in melatonin supplemented Cucumis sativus through modulation of antioxidative defense system. Iranian Journal of Plant Physiology 10, 3135-3154. https://doi.org/10.1016/j.scienta.2020.109203

Shah, T., Khan, Z., et al., 2023. Alleviation of cadmium toxicity in wheat by strigolactone: Regulating cadmium uptake, nitric oxide signaling, and genes encoding antioxidant defense system. Plant Physiology and Biochemistry 202, 107916. https://doi.org/10.1016/j.plaphy.2023.107916

Song, C., Manzoor, M.A., et al., 2024. Photosynthetic machinery and antioxidant enzymes system regulation confers cadmium stress tolerance to tomato seedlings pretreated with melatonin. Scientia Horticulturae 323, 112550. https://doi.org/10.1016/j.scienta.2023.112550

Teng, Y., Guan, W., et al., 2022. Exogenous melatonin improves cadmium tolerance in *Solanum nigrum* L. without affecting its remediation potential. International Journal of Phytoremediation 24, 1284-1291. https://doi.org/10.1080/15226514.2021.2025204

Tousi, S., Zoufan, P., et al., 2020. Alleviation of cadmium-induced phytotoxicity and growth improvement by exogenous melatonin pretreatment in mallow (*Malva parviflora*) plants. Ecotoxicology and Environmental Safety 206, 111403. https://doi.org/10.1016/j.ecoenv.2020.111403

Umapathi, M., Kalarani, M.K., et al., 2022. Alleviation of cadmium phytotoxicity through melatonin modulated physiological functions, antioxidants, and metabolites in tomato (*Solanum lycopersicum* L.). BioMetals 35, 1113-1132. https://doi.org/10.1007/s10534-022-00428-y

Wang, M., Duan, S., et al., 2019. Foliar spraying of melatonin confers cadmium tolerance in *Nicotiana tabacum* L. Ecotoxicology and Environmental Safety 170, 68-76. https://doi.org/10.1016/j.ecoenv.2018.11.127

Wang, T., Song, J., et al., 2021. Melatonin alleviates cadmium toxicity by reducing nitric oxide accumulation and IRT1 expression in Chinese cabbage seedlings. Environmental Science and Pollution Research 28, 15394-15405. https://doi.org/10.1007/s11356-020-11689-w

Wang, X., Chen, S., et al., 2010. Desiccation tolerance mechanism in resurrection fern-ally Selaginella tamariscina revealed by physiological and proteomic analysis. Journal of Proteome Research 9, 6561-6577. https://doi.org/10.1021/pr100767k

Wei, Z., Li, C., et al., 2019. Melatonin increases the performance of *Malus hupehensis* after UV-B exposure. Plant Physiology and Biochemistry 139, 630-641. https://doi.org/10.1016/j.plaphy.2019.04.026

Wu, S., Wang, Y., et al., 2021. Exogenous melatonin improves physiological characteristics and promotes growth of strawberry seedlings under cadmium stress. Horticultural Plant Journal 7, 13-22. https://doi.org/10.1016/j.hpj.2020.06.002

Xu, L., Zhang, F., et al., 2020. Melatonin confers cadmium tolerance by modulating critical heavy metal chelators and transporters in radish plants. Journal of Pineal Research 69, e12659. https://doi.org/10.1111/jpi.12659

Yang, H., Fang, R., et al., 2023. Potential roles of melatonin in mitigating the heavy metals toxicity in horticultural plants. Scientia Horticulturae 321, 112269. https://doi.org/10.1016/j.scienta.2023.112269

Yin, Z., Lu, J., et al., 2019. Exogenous melatonin improves salt tolerance in tomato by regulating photosynthetic electron flux and the ascorbate–glutathione cycle. Journal of Plant Interactions 14, 453-463. https://doi.org/10.1080/17429145.2019.1645895

Zhang, N., Sun, Q., et al., 2016. Melatonin improved anthocyanin accumulation by regulating gene expressions and resulted in high reactive oxygen species scavenging capacity in cabbage. Frontiers in Plant Science 7, 197. https://doi.org/10.3389/fpls.2016.00197

Zhang, Y., Liang, Y., et al., 2019. Silicon compensates phosphorus deficit-induced growth inhibition by improving photosynthetic capacity, antioxidant potential, and nutrient homeostasis in tomato. Agronomy 9, 733. https://doi.org/10.3390/agronomy9110733

Zhang, Y., Yang, C., et al., 2024. Alleviation of salt toxicity by melatonin supplementation in eggplant involves the up-regulation of ascorbate–glutathione and glyoxalase system. Scientia Horticulturae 326, 112720. https://doi.org/10.1016/j.scienta.2023.112720

Zhou, H., Cheng, L., et al., 2023. Current understanding of unlocking the power of melatonin and other phytohormones to boost abiotic stress tolerance in solanaceous vegetable crops. Scientia Horticulturae 321, 112313. https://doi.org/10.1016/j.scienta.2023.112313