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Biochemical and morphological adaptations of *Ficus virens* under cadmium-induced environmental stress conditions

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Abstract

Cadmium (Cd), a prevalent heavy metal pollutant, poses substantial threats to plant health, manifesting in altered growth and disrupted biochemical processes. This study investigates the growth behavior and biochemical responses of *Ficus virens* under cadmium stress. A controlled pot trial was conducted at the Forest Experimental Nursery, Institute of Forest Science, Baghdad-ul-Jadeed Campus, Islamia University of Bahawalpur. Six-month-old *Ficus virens* plants, uniform in size, age, and health, were transplanted into pots and subjected to varying concentrations of Cd: T1 (control), T2 (5 ppm), T3 (10 ppm), T4 (15 ppm), and T5 (20 ppm). Results indicated that plant growth was optimal at T1 (control) and significantly inhibited at T5 (20 ppm). Biochemical analysis revealed maximum levels of SOD, POD, CAT, and MDA (18.49 mg g^{-1} protein, 0.954 mg g^{-1} protein, 41.60 mg g^{-1} protein, and 6.13 mg g^{-1} protein, respectively) at T5, and minimum levels (1.94 mg g^{-1} protein, 0.285 mg g^{-1} protein, 3.05 mg g^{-1} protein, and 1.88 mg g^{-1} protein, respectively) at T1. Photosynthetic parameters, including Chl a, Chl b, total Chl, and carotenoids, were highest in the control group (1.73 mg g^{-1} FW, 1.80 mg g^{-1} FW, 1.56 mg g^{-1} FW, and 0.93 mg g^{-1} FW, respectively) and lowest at T5 (0.81 mg g^{-1} FW, 0.75 mg g^{-1} FW, 1.56 mg g^{-1} FW, and 0.23 mg g^{-1} FW, respectively).

1. Introduction

Ficus virens, commonly known as the White Fig or Pilkhan tree, is a prominent species of evergreen tree belonging to the Moraceae family, native to South Asia, present countries such as India, Sri Lanka, Nepal, and Bangladesh (Patel *et al*., 2023). This species thrives in a diverse range of environments, from tropical rainforests to urban areas, making it a significant ecological and cultural asset across its native range. The distinctive glossy green leaves of *Ficus virens* provide ample shade, making it a popular choice for decorative purposes in parks and gardens(Orabi and Orabi, 2016) . Its small, pear-shaped fruits, turning light yellow or white upon

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maturation, are not only aesthetically pleasing but also serve as a vital food source for numerous animal species, contributing to its ecological importance (Kunkel, 2012).

Beyond its ecological and aesthetic value, *Ficus virens* holds considerable medicinal significance in South Asian herbal medicine traditions. Various parts of the tree, including leaves, bark, and latex, have been utilized for centuries to treat ailments ranging from respiratory problems to digestive disorders(Salehi *et al*., 2021). In addition to its ecological and medicinal roles, *Ficus virens* plays a crucial cultural role among indigenous communities, where it is often revered as sacred and incorporated into ceremonies and customs (Aumeeruddy-Thomas and Hossaert-McKey, 2024). *Ficus virens* exhibits remarkable ecological flexibility, thriving in diverse habitats such as savannas, coastal ecosystems, tropical dry forests, and rainforests across Asia and Oceania (McCoy, 2010). Its ability to colonize different environments and elevations is supported by mutualistic interactions with nitrogen-fixing bacteria in its roots, which enhance soil fertility and benefit neighboring plants(Ehis-Eriakha and Adetunji, 2022).

However, the growth and sustainability of *Ficus virens* are increasingly threatened by environmental factors such as climate change and heavy metal pollution. Studies have shown that variations in temperature, humidity, and soil composition significantly influence its vegetative growth and reproductive success (Chaudhary and Rathore, 2019). Moreover, urbanization and industrial activities introduce heavy metals like lead, cadmium, and zinc into *Ficus virens* habitats, posing serious threats to its physiological processes and overall health (Zheng *et al*. 2020). Understanding the responses of *Ficus virens* to these environmental stressors is critical for its conservation and sustainable utilization. This study aimed to investigate the impact of cadmium contamination on the growth behavior, biochemical responses, and potential phytoremediation capabilities of *Ficus virens*. By elucidating these aspects, we seek to contribute valuable insights into the ecological resilience and conservation strategies for this important tree species.

2. Materials and Methods

2.1 Study Site and Planting Material

The experiment was conducted at the Forest Nursery, Institute of Forest Science, Baghdad-ul-Jaded Campus, Islamia University of Bahawalpur. Disease-free and uniform-size six-month-old seedlings of *Ficus virens* were sourced from the Forest Nursery and Experimental Area. In November 2022, these seedlings were transplanted into pots (13 x 17 cm) filled with 2 kg of soil media. After transplantation, the pots were watered and placed in a controlled environment.

2.2 Climatic Conditions

Bahawalpur's climate is characterized by high temperatures, high evaporation rates, low relative humidity, infrequent rainfall, and strong summer winds. The annual rainfall ranges between 90-200 mm, and temperatures vary from -20 to 50°C.

2.3 Experimental Layout and Treatment Application

The experiment included five treatments with six replicates each:

1. T1 (Control)
2. T2 (5 ppm)
3. T3 (10 ppm)
4. T4 (15 ppm)
5. T5 (20 ppm)

The treatments were arranged in a completely randomized block design. Cadmium stress was applied in three doses.

2.4 Measurement of Growth Parameters

During seedling establishment and before harvest, plant height and stem diameter were measured using digital calipers and tailor tape. After harvesting, shoot and root portions were weighed using an electronic scale (JJ3000B), followed by drying in a thermal electric thermostat drying oven (DGH - 9202 series) at 74°C until constant weight.

2.5 Determination of Photosynthetic Pigments

Photosynthetic pigments (chlorophylls and carotenoids) were determined using the methods described by Amon (1949) and Davis (1986). Leaf discs were extracted with 80% acetone, and pigment concentrations were measured spectrophotometrically at specific wavelengths.

2.6 Extraction Preparation for Biochemical Parameters

Proline, soluble protein, and antioxidant enzyme (Peroxidase, Superoxide Dismutase, Catalase) extractions followed the guidelines of Naqvi et al. (2011). Samples were homogenized in phosphate buffer, filtered, and centrifuged before storage at -80°C for subsequent analysis.

2.7 Determination of Peroxidase

Peroxidase (POD) activity was measured following the method of Liu and Huang (2000), using guaiacol as a substrate and measuring absorbance changes at 470 nm.

2.8 Determination of Superoxide Dismutase

Superoxide Dismutase (SOD) activity was assessed using the Giannopolitis and Ries (1977) method, measuring inhibition of NBT photoreduction at 560 nm.

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2.9 Determination of Catalase

Catalase (CAT) activity was determined according to Liu et al. (2009), based on the decomposition of H_2O_2 at 240 nm.

2.10 Determination of Soluble Protein

Soluble protein content was quantified using the Bradford method (Bradford, 1976), measuring absorbance at 595 nm and referencing a standard curve of bovine serum albumin (BSA).

2.11 Statistical analysis

The data of each was analyzed using one-way ANOVA for species effect (Statistica 12.1, Maison Alford, France). Means were compared using Tukey's HSD test and all tests were taken as significant at $P< 0.05$ and were expressed with their standard error.

3. Results

3.1 Growth Parameters.

The plant height of *Ficus virens* varied significantly under Cd stress. The tallest plants (66.23 cm) were observed in T1 (control), whereas the shortest (44.3 cm) were in T5 (20 ppm). As Cd concentration increased, plant height decreased linearly, with a treatment response order of T1 > T2 > T3 > T4 > T5. Stem diameter exhibited significant variation under Cd stress. Maximum diameter (14.63 mm) was recorded in T1 (control), decreasing to 7.95 mm in T5 (20 ppm) with increasing Cd levels. *Ficus virens* showed significant variation in shoot fresh weight under Cd stress. The highest weight (71.50 g) was in T1, decreasing to 39.66 g in T5, reflecting a decrease with increasing Cd concentration. There was significant variation in shoot dry weight under Cd stress. The highest dry weight (42.50 g) was in T1, decreasing to 13.50 g in T5 as Cd concentration increased. Root fresh weight exhibited significant variation under Cd stress. Maximum weight (57.33 g) was in T1, decreasing to 35.66 g in T5 with increasing Cd concentration. Root dry weight showed significant variation under Cd stress. The highest dry weight (40.50 g) was in T1, decreasing to 17.66 g in T5 as Cd levels increased. Total biomass varied significantly under Cd stress. Maximum biomass (83.00 g) was in T1, decreasing to 28.50 g in T5 with increasing Cd concentration. Results of growth parameters are shown in Figure 3.1.

3.2 Biochemical Parameters

SOD activity showed significant variation under Cd stress. The lowest activity (1.94 mg_1 protein) was in T1, increasing to 18.49 mg_1 protein in T5 with increasing Cd concentration. *POD* activity exhibited significant variation under Cd stress. The lowest activity (0.285 mg_1 protein) was in T1, increasing to 0.954 mg_1 protein in T5 as Cd concentration increased. *CAT* activity showed significant variation under Cd stress. The lowest activity (3.05 mg_1 protein) was in T1, increasing to 41.60 mg_1 protein in T5 with increasing Cd levels. *MDA* content varied significantly under Cd stress. The lowest content (1.88 mol g_1 FW) was in T1, increasing to 6.13 mol g_1 FW in T5 as Cd concentration increased. Total soluble protein content showed significant variation under Cd stress. Maximum protein content (5.78 mg g_1 FW) was in T1, decreasing to 1.51 mg g_1 FW in T5 with increasing Cd concentration. Figure 3.2 shows the biochemical activity of various enzymes.

3.3 Photosynthetic pigments

Chl a content varied significantly under Cd stress. Maximum content (1.73 mg g_1 FW) was in T1, decreasing to 0.81 mg g_1 FW in T5 with increasing Cd concentration. *Chl b* content exhibited significant variation under Cd stress (Figure 3.14). Maximum content (1.80 mg g_1 FW) was in T1, decreasing to 0.75 mg g_1 FW in T5 with increasing Cd concentration. Total chlorophyll content varied significantly under Cd stress (Figure 3.15). Maximum content (3.54 mg g_1 FW) was in T1, decreasing to 1.56 mg g_1 FW in T5 as Cd concentration increased. *Carotenoid* content showed significant variation under Cd stress. Maximum content (0.93 mg g_1 FW) was in T1, decreasing to 0.23 mg g_1 FW in T5 with increasing Cd levels.

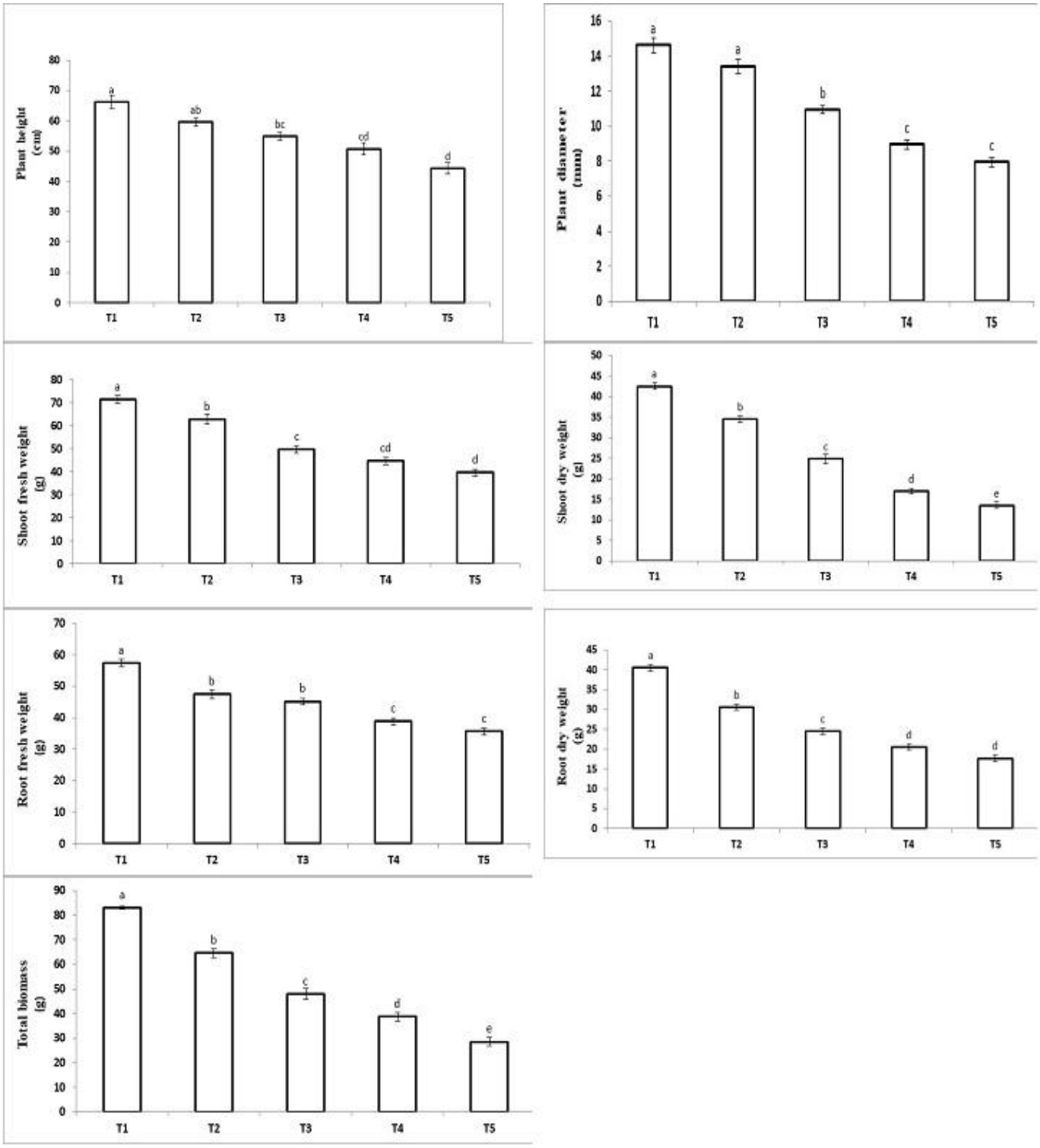


Figure 3.1: Growth potential of *Ficus virens* under cadmium stress

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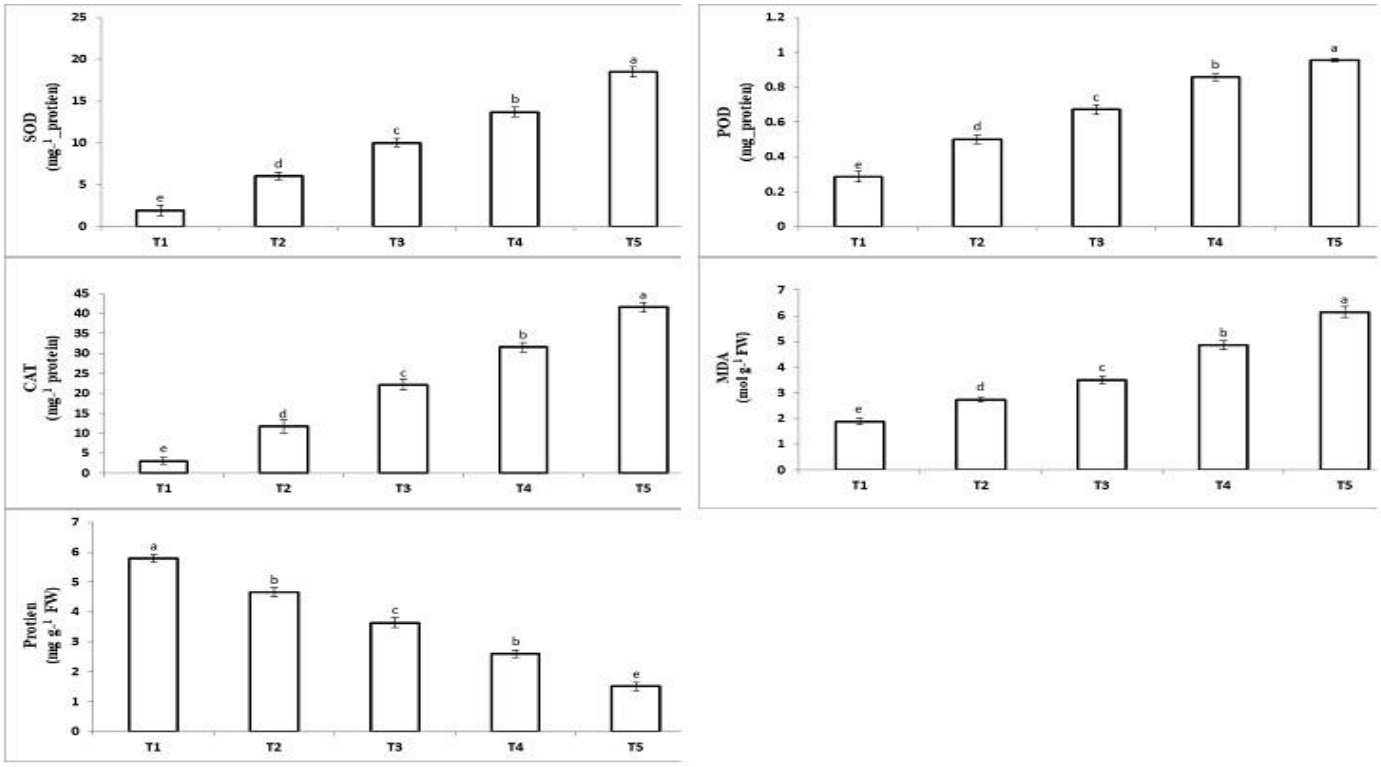


Figure 3.2: Biochemical response of *Ficus virens* under cadmium stress

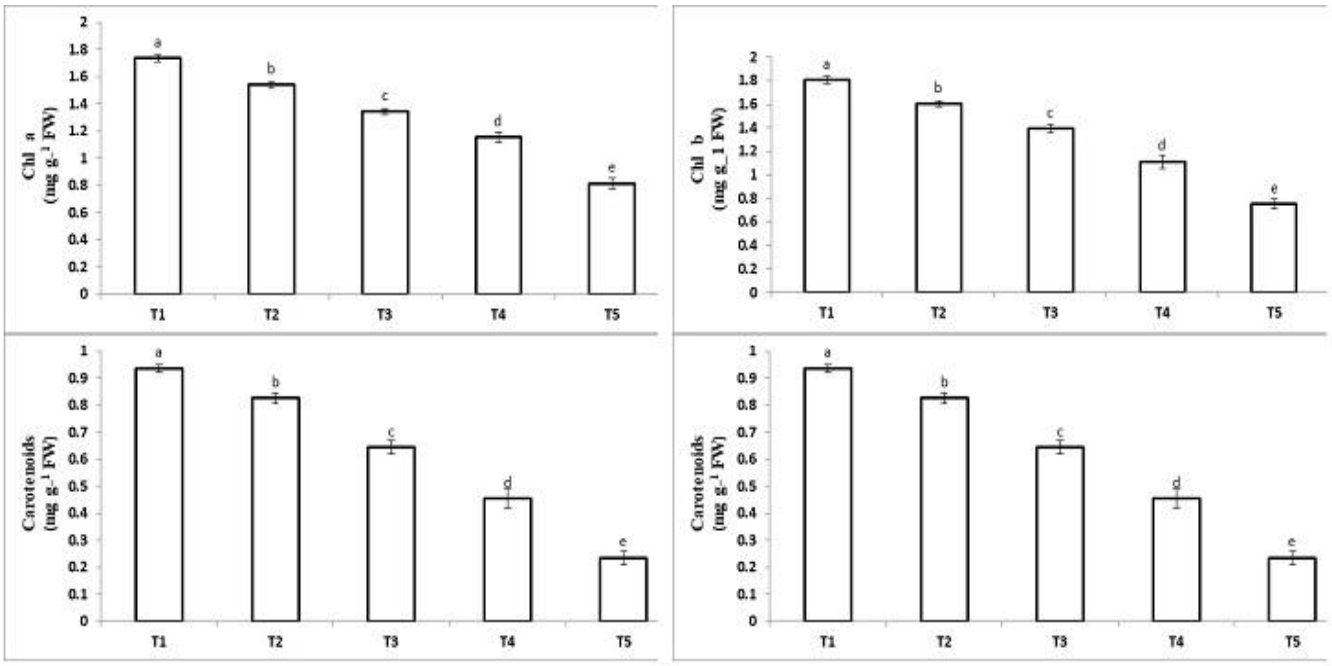


Figure 3.3: Photosynthetic activity of *Ficus Virens* under cadmium stress

4. Discussion

4.1 Impact on Plant Growth

The exposure to cadmium (Cd) significantly inhibited the growth of *Ficus virens*, with higher Cd concentrations correlating with a marked reduction in plant height, diameter, shoot fresh and dry weight, root fresh and dry weight, and overall biomass. These results indicate that cadmium pollution can severely impact the growth and general development of *Ficus virens*. The reduction in plant height observed in *Ficus virens* exposed to increasing doses of Cd aligns with previous studies on the phytotoxic effects of heavy metals on various plant species (Gyawali *et al*., 2013). The inhibition of cell extension and division, disruption of cell wall formation, and impaired nutrient and water uptake are possible mechanisms through which Cd affects plant height (Farid *et al*., 2013). Oxidative stress caused by reactive oxygen species (ROS) accumulation further exacerbates these negative effects, leading to reduced growth and vigor in *Ficus virens* (Mandal *et al*., 2023).

A modest decrease in stem diameter was noted at lower cadmium concentrations, consistent with research on wheat plants (Hussain *et al*., 2021). At higher Cd doses, a more pronounced reduction in stem diameter was observed, supporting findings by (Zhang *et al*., 2021) in *Brassica juncea*. The cytotoxicity and disruption of physiological processes caused by Cd accumulation in plant tissues likely contribute to this decline. While *Ficus virens* may tolerate low Cd levels to some extent, higher concentrations lead to significant growth impairment (Li *et al*., 2023). Shoot fresh and dry weight also significantly decreased with increasing soil cadmium content. This outcome is consistent with studies on the detrimental effects of Cd on plant development (Shah *et al*., 2011). Cd uptake by plant roots and its subsequent accumulation in tissues disrupts critical functions such as photosynthesis, nitrogen absorption, and water transport, leading to reduced biomass accumulation (Haider *et al*., 2021).

4.2 Effect on Leaf Functioning

The study found a significant increase in the production of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and peroxidase (POX), as well as malondialdehyde (MDA), with increasing cadmium concentration. This indicates that the antioxidant defense systems of *Ficus virens* are activated to combat Cd-induced oxidative stress. Increased CAT activity in response to Cd stress suggests an adaptive defense mechanism against oxidative damage (Cuypers *et al*., 2010). CAT plays a crucial role in maintaining cellular redox balance by converting hydrogen peroxide into water and oxygen (Ighodaro & Akinloye, 2018). Similarly, enhanced SOD activity, which dismutates superoxide radicals into less harmful molecules, highlights the plant's response to elevated ROS levels caused by Cd exposure (Gill *et al*., 2015). The overexpression of SOD helps mitigate oxidative damage, preserving the plant's physiological functions (Foyer and Shigeoka, 2011).

The increase in POX activity further supports the role of antioxidant enzymes in detoxifying ROS produced under Cd stress (Dey *et al*., 2007). Enhanced POX production may be a defense mechanism against Cd-induced oxidative damage, with a dose-dependent effect observed at higher Cd concentrations (Požgajová *et al.*, 2022). Elevated MDA levels in response to Cd exposure indicate significant oxidative stress and lipid peroxidation, compromising cell membrane integrity (Chen *et al*., 2014). The increase in MDA levels suggests severe oxidative damage to cellular components, with the plant's antioxidant defenses being activated to mitigate this damage (Chen *et al*., 2015).

4.3 Effect on Photosynthetic Pigments

The study also revealed a decrease in the synthesis of photosynthetic pigments, including chlorophyll a, chlorophyll b, and carotenoids, with increasing cadmium concentration. Reduced chlorophyll levels indicate lower photosynthetic efficiency, further hampering the growth of *Ficus virens*. The decline in chlorophyll a concentration under Cd stress affects the plant's ability to perform photosynthesis, leading to reduced carbon dioxide uptake and energy production (Chen e*t al*., 2015). Disruption of the chlorophyll biosynthesis pathway by Cd and oxidative stress-induced chlorophyll degradation are potential mechanisms for this reduction (Grobelak *et al*., 2019). A similar reduction in chlorophyll b and total chlorophyll content highlights the overall vulnerability of photosynthetic pigments to heavy metal stress. The decreased chlorophyll levels suggest that *Ficus virens* may have a reduced capacity for photosynthetic activity in Cd-contaminated environments, raising concerns about its effectiveness in ecological restoration efforts (Vimal *et al*., 2024). The decrease in carotenoid synthesis under Cd exposure further impacts the plant's ability to mitigate oxidative stress and photodamage (Zhang *et al*., 2020). Reduced carotenoid levels may also affect the plant's nutritional value and its interactions with herbivores and other trophic levels in the ecosystem (Bassman, 2004).

Conclusion

In this study, we examined the impact of cadmium contamination on the growth and biochemical responses of *Ficus virens*, revealing severe detrimental effects. Cadmium exposure significantly hindered growth parameters such as plant height, stem diameter, and total biomass. Biochemical analyses indicated oxidative stress, shown by decreased chlorophyll content and elevated antioxidant enzyme activities. These findings highlight the necessity of monitoring and mitigating cadmium contamination to protect *Ficus virens* in polluted environments. Future research should delve into the molecular mechanisms of cadmium stress responses and explore the species' potential in phytoremediation. Our study underscores the urgent need to address heavy metal pollution to ensure the conservation of this vital species.

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