



Ameliorative Role of Poultry Manure and Exogenous Absciscic Acid in Mitigating Cadmium Toxicity in Wheat (*Triticum aestivum* L.)

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Abstract

Cadmium (Cd) contamination is one of the most important environmental problems which has a negative impact on wheat (*Triticum aestivum* L.) development, physiology and yield formation. To assess the ability of poultry manure (PM) and exogenous application of abscisic acid (ABA) to reduce Cd stress in wheat, a pot experiment was conducted. The experiment included seven treatments: T1, untreated control; T2, Cd stress; T3, PM (1%); T4, ABA (10 µM); T5, Cd + PM; T6, Cd + ABA; and T7, Cd + PM + ABA. It was observed that hydrogen peroxide (H₂O₂) content was increased by Cd stress in the plants to about 118 µM g⁻¹ FW (fresh weight) while in the control plants it was 42 µM g⁻¹ FW, which is about 181% of the control. The concentration of malondialdehyde (MDA) rose from about 16 to 27 µM g⁻¹ FW. Cadmium exposure also caused a decrease in tiller number (from about 5.2 to 3.6 tiller/plant), spike length (from 8.2 to 5.7 cm), spikelets per spike (from 12.5 to 8.7 spikelets/spike) and grains per spike (from 10.2 to 7.1 grains/spike). In combined treatments (Cd + PM + ABA), the decrease in concentration was observed for H₂O₂ to ~ 90 µM g⁻¹ FW and for MDA to ~ 17 µM g⁻¹ FW. Tiller number, spike length and spikelets per spike were increased to ~ 4.7, 7.5 cm, and 11.3, respectively, while the number of grains per spike was not as improved and was ~ 9.3. These results showed that PM and ABA were partially able to reduce Cd-induced oxidative stress and enhanced yield-related traits in wheat. Further research is needed to evaluate soil Cd availability, grain accumulation of Cd, activities of antioxidant enzymes, and final grain yield, to verify treatment effectiveness and food-safety implications.

Keywords: Absciscic acid, Cadmium stress, Hydrogen peroxide, Malondialdehyde, Poultry manure, Wheat, oxidative stress, Yield attributes.

1. Introduction

Wheat is one of the most important cereal crops in the world, and it has a central role in food security globally for its carbohydrates, proteins and essential nutrients. Being widely grown in different agroecological zones, it is used for making flour, bread, chapati, pasta, and other foods (Etaware et al., 2025). Wheat is one of the most important Rabi crops in Pakistan which meets national food needs, employment and livelihood needs of farmers. But the productivity and quality of wheat is becoming a new challenge due to environmental stresses, soil fertility reduction and contamination of agricultural lands with potentially toxic heavy metals. In this group, cadmium (Cd) is of major concern as it can affect plant growth, biochemistry and physiology, and stress plant edible parts (Majeed et al., 2021). Thus, the presence of Cd in wheat is an important issue related to agriculture and food safety that

needs proper and sustainable management strategies (Rizwan et al., 2016). Cadmium is a non-essential heavy metal that can be introduced to agricultural soils via industrial emissions, mining, wastewater irrigation, atmospheric deposition, and the re-application of some fertilizers and organic amendments. It is influenced by soil properties like organic matter, pH, cation exchange capacity and soil texture (Çatav et al., 2020). The wheat can uptake Cd from polluted soils and the metal can be accumulated in the root or translocated to the aerial parts of the plant, such as leaves and grain. High levels of Cd can affect crop quality and lead to a higher risk of exposure to humans via wheat containing foods. Cd toxicity was previously shown to reduce the germination rate, root and shoot growth, mineral nutrient uptake, photosynthesis, and grain yield of wheat. Additionally, the Cd uptake, accumulation, and transport capacity of wheat cultivars vary, which is influenced by both cultivar and growth conditions and must be considered when managing Cd (Zhou & Li, 2022).

Cadmium toxicity has a negative effect on wheat growth, due to the interference of root growth, water uptake and the uptake and distribution of essential nutrients. Root growth is particularly significant due to the role of roots in anchoring plants and absorbing water and mineral nutrients. Cd exposure can cause root growth inhibition, root cell damage and disrupt nutrient movement, which can result in reduced shoot growth and biomass production. It is also capable of interfering with the absorption of K, Ca, Mg, Fe, and other essential nutrients for plant metabolism. The result of these changes may be the decrease in leaf growth, the height of the shoots and fresh and dry weight of the plants (Mushtaq et al., 2025). Level of Cd toxicity varies with the concentration of Cd, exposure time, developmental stage of plants, soil conditions, and the genetic makeup of the wheat cultivar. Therefore, wheat shoot length, root length, leaf number and shoot and root biomass are good parameters to assess wheat response to Cd stress and the effectiveness of ameliorative treatments (Zheng et al., 2023). Cadmium also has an impact on physiological processes involved in photosynthesis and plant water relations. It can cause a decrease in chlorophyll concentration, affect the functioning of the chloroplast and disrupt photosynthetic machinery, leading to a decrease in carbon assimilation and growth. Other changes in leaf area can reduce the amount of photosynthetically active radiation intercepted. Moreover, the effect of Cd on water uptake and on cellular membranes can lead to a decreased relative water content and membrane stability. Combination of decreased photosynthesis, nutrient imbalance, and altered water relations may result in decreased plant vigor and biomass (Li et al., 2023). Hence, the assessment of chlorophyll content, leaf area, relative water content and membrane stability can be useful in understanding the physiological status under Cd stress in wheat and the alleviation of stress-induced damages by poultry manure and ABA treatments (Pokovai et al., 2025). Although reactive oxygen species (ROS) are formed in plants during metabolic processes, under stress conditions, the levels of ROS may be high enough to damage cellular membranes, proteins, pigments and other biological components. Hydrogen peroxide (H_2O_2) is frequently used as an index of oxidative stress, and malondialdehyde (MDA) as an index of lipid peroxidation and membrane damage. Enzymes such as Superoxide dismutase (SOD), Catalase (CAT), Peroxidase (POD) and Ascorbate peroxidase (APX) are all involved in the antioxidant activity of plants. SOD converts superoxide radicals to hydrogen peroxide, and CAT, POD and APX are responsible for removing hydrogen peroxide. Wheat is a crop that has been used in several studies to reveal changes in antioxidant enzyme activities and in MDA concentrations following Cd exposure, but the extent of the changes varies between cultivars and is dependent on the cultivar's tolerance mechanism. Therefore, measuring H_2O_2 , MDA and antioxidant enzyme activities can help determine if a treatment can enhance wheat plant ability to cope with Cd-induced oxidative stress (Çatav et al., 2020; Zhao et al., 2019; Zhou and Li, 2022).

Abscissic acid (ABA) is a naturally occurring plant growth hormone, which functions in many aspects of plant growth, such as stomatal movement, water relations and responses to environmental stresses. ABA signaling can be used to regulate stomatal closure, minimize unnecessary water loss, and control stress response gene expression under stress conditions. It also is involved in hormonal signaling interactions, antioxidant defense and other protective mechanisms that enable plants to respond to negative environmental factors. The significance of ABA in plant adaptation to abiotic stresses such as salinity, drought, temperature extremes, heavy metal tolerance etc. has been recognized in previous reviews (Vishwakarma et al., 2017; Bharath et al., 2021). Poultry manure is an organic amendment which may enhance the fertility of the soil by providing the essential nutrients and organic matter. It has potential to enhance the soil structure, water holding capacity, soil microbial activity and nutrient cycling which can favor root growth and crop development. Changes in soil pH, adsorption of Cd in soil, formation of complexes, and other soil chemical processes may also be affected by the use of organic amendments, which in turn may affect Cd mobility and bioavailability. These effects can lead to changes in plant root uptake and transport to the aboveground tissues of Cd. However, poultry manure is not always effective at decreasing Cd levels. Its impact is related to the initial soil characteristics, manure components, application rate and level of contamination. In a meta-analysis of livestock and poultry manure application, Tang et al. (2024) also found that the effects of manure application on Cd concentrations in soil and crop were inconsistent, with manure application potentially also leading to higher Cd concentrations in soil and crops under certain conditions. Therefore, poultry manure is not a general remedy for Cd immobilization but should be used as a nutrient source and as a potential Cd immobilizer.

This integrated management of manure and exogenous ABA could be complementary in wheat grown under Cd stress. The manure of poultry affects fertility and the soil environment, while ABA regulates the physiological responses of plants and their stress signaling. They can be used together to maintain root and shoot growth, acquisition of nutrients, preservation of photosynthetic pigment and antioxidant defense. In a recent wheat study, combining biochar with ABA resulted in an improvement in growth and root morphology, a decrease in Cd accumulation, and a decrease in oxidative injury, thereby offering relevant evidence for the beneficial use of ABA in combination with a soil amendment, such as biochar. Biochar and poultry manure properties, however, are different and the above findings cannot be directly used to confirm the effectiveness of the poultry manure and ABA mixture (Khaliq et al., 2024). Even though there is a growing interest in using organic amendments and plant hormones for the management of heavy metal stress, more studies are required to understand the effects of poultry manure and exogenous ABA alone and in combination in wheat under Cd stress. Specifically, their activity should be investigated in plant growth, root architecture, photosynthetic pigments, leaf area, relative water content, membrane stability, oxidative stress indicators, antioxidant enzyme activities, and accumulation of Cd in plant

tissues. An integrated approach can help to identify differences between the improvement of plant growth and actual changes of the uptake and distribution of Cd. So the present study was planned to explore individual and synergistic effect of poultry manure and exogenous ABA in wheat under Cd stress. The parameters measured in the study were shoot length, root length, number of leaves, fresh biomass, dry biomass, root surface area, root diameter, root volume, root tips, chlorophyll content, leaf area, relative water content, membrane stability index, activities of SOD, CAT, POD, and APX. The accumulation of cadmium in roots and shoots was also investigated. Based on this assumption, the effect of poultry manure and ABA alone and in combination on wheat performance under Cd stress was hypothesized through their involvement in plant growth, physiological functioning and strengthening of the plant antioxidant defense system, and possible roles in Cd accumulation. The results could provide insights into the benefit and limitations of these treatments as a management approach to enhance wheat performance in contaminated soils.

2. Materials and Methods

2.1. Experimental Site and Growth Conditions

A pot experiment was thus carried out in a rain protected wire house at the Reed Pakistan Farm Area, Pakistan to understand the ameliorative effect of poultry manure and exogenous abscisic acid (ABA) on cadmium (Cd) toxicity in wheat. Seeds of wheat were procured from a trusted agricultural seed supplier that provided healthy and uniform wheat seeds. Good germination and crop uniformity was ensured by careful selection. Seeds were planted at an appropriate planting depth and 8kg of well-mixed soil was used per pot. Poultry manure 1% was added to the designated treatments prior to sowing. The concentration of cadmium 2µg/kg was applied at the predetermined concentration, and ABA was exogenously applied to the treatments at 10 µmol L⁻¹. The treatments applied in the experiment were: T1 (control), T2 (Cd), T3 (ABA), T4 (Cd + ABA), T5 (poultry manure), T6 (Cd + poultry manure), T7 (poultry manure + ABA) and T8 (Cd + poultry manure + ABA). Three replications of each treatment were performed in a completely randomized design (CRD). Upon germination, uniform and healthy seedlings were left in each pot. The irrigation was applied even, and soil moisture was kept at proper level during the whole period of experiment. Mowing was used to control weeds as needed and other normal crop management was conducted. The effectiveness of poultry manure and ABA mitigating Cd toxicity was evaluated using plant growth, physiological and biochemical attributes, antioxidant enzyme activities and accumulation of Cd in wheat tissues (Akbar et al., 2025; Gomez and Gomez, 1984; Sikka et al., 2025; Ugwu et al., 2025).

2.2. Growth Parameter Analysis

2.2.1. Measurement of Growth Parameters

Wheat plant height, root length and shoot length were recorded at harvesting with the help of the meter scale. The fresh weight of the shoots and roots was taken on a digital weighing balance. The samples were then oven dried to constant weight for determination of dry biomass. When available, root attributes such as root length, surface area, volume and average diameter were determined with a root-scanning system (WinRHIZO Pro, Regent Instruments, Canada).

2.2.2. Chlorophyll Content and Leaf Area

The chlorophyll content of the leaves was determined with a chlorophyll meter (SPAD-502, Minolta, Japan). Leaves of wheat under the same growth stage were fully expanded and measured. Leaf area measurement was done using a leaf area meter or appropriate image-analysis program.

2.2.3. Relative Water Content (RWC)

The method described by Lazcano-Ferrat and Lovatt (1999) was used to determine the relative water content. Fresh leaf weight (FW) was measured as soon as the samples were taken. The leaves were first placed in distilled water for about 24 hours to get the turgid weight (TW). Dry weight (DW) was then calculated after oven drying at 60 °C until constant weight of the samples. The following formula was used in the calculation of RWC:

$$\text{RWC}\% = (\text{FW} - \text{DW}) / (\text{TW} - \text{DW}) \times 100$$

2.2.4. Membrane Stability Index (MSI)

The stability of the membrane was assessed by the method of Sairam and Saxena 2000. Fresh wheat leaf samples (0.1 g) taken from each treatment were transferred to test tubes and distilled water and kept in water bath at 40 °C for 30 minutes. Initial electrical conductivity (C₁) was measured. The samples were then heated at 100 °C for 10 minutes, cooled to room temperature and the final electrical conductivity (C₂) was determined.

MSI was calculated using the following formula:

$$\text{Membrane Stability Index} = [1 - (C_1 / C_2)] \times 100$$

2.2.5. Determination of Oxidative Stress Indicator

The estimation of lipid peroxidation was done by the method prescribed by Jambunathan (2010). Wheat leaves were homogenized in 0.1% trichloroacetic acid (TCA) solution (0.25 g). Homogenate was centrifuged for 15 minutes at 10,000 × g. The supernatant was then treated with thiobarbituric acid (TBA) and absorbance was determined at 532 and 600 nm to subtract out any non-specific turbidity. The extinction coefficient of 155 mM⁻¹cm⁻¹ was used to calculate the content of MDA. Hydrogen peroxide (H₂O₂): The content of hydrogen peroxide was determined according to method of Velikova et al., 2000. Fresh leaf tissue (0.2 g) was homogenized in 1 mL of 0.1% TCA and then centrifuged at 8,000 × g for 15 min. The supernatant (0.5 mL) was mixed with 0.5 mL of 10 mM potassium phosphate buffer and 1 mL of 1 M potassium iodide. Absorbance at 390 nm was determined and the content of H₂O₂ was quantified by using the appropriate calibration.

2.2.6. Determination of Antioxidant Enzyme

Leaf discs (500 mg) were collected and at 0-4 °C, they were homogenate with 10 ml of an extraction buffer solution containing 1 g of polyvinyl pyrrolidone (PVP), 1 mM EDTA and 0.5% Triton X-100 (Kamran et al., 2019a). The homogenate was centrifuged at 12,000 rpm for 20 min and supernatant (2–3 ml) was isolated and used for enzyme assays. The Superoxide Dismutase (SOD) activity of the supernatant that was isolated was determined by NBT technique, and absorbance was measured at 560nm (Giannopolitis and Ries 1977). The peroxidase (POD) activities were measured by guaiacol and H₂O₂ and KH₂PO₄ buffer with EDTA (pH 7) then calculated from a spectrophotometer at 470 nm and catalase (CAT) at 240 nm (Chance and Maehly 1955). The Ascorbate Peroxidase (APX) activity was measured at 290 nm with the use of potassium phosphate buffer with EDTA and extract (Prochazkova et al., 2001).

2.2.7. Grain Yield and Yield components

The number tillers per plant counted and recorded. Ten randomly selected spikes were measured with a meter rod and the mean spike length in centimeters estimated. Each sub-plot spikes chosen randomly and the number of spikelets/spikes was counted and averaged. Haphazardly selected spikes were separated manually, their grains were calculated and mean weight was documented (Ullah et al., 2018).

2.3. Statistical Analysis

The data were analyzed and interpreted by the Statistics programme Statistix 8.1 (Statistics, IL, USA) and the results are presented in tables and graphs. The standard deviations were used as the error bars and the graph bars indicate the average value of three replicates; the source of variation was analyzed and examined using Least Significant Difference (LSD) test at 0.05 level of significance (Steel et al., 2000).

3. Results and Discussion

3.1. Interactive Effect of Poultry Manure and Absciscic Acid under Cadmium Stress on Growth Attributes of Wheat

As shown in the figure 1, after cadmium (Cd) stress the growth and biomass production of wheat was reduced while the growth attributes were improved by the application of poultry manure (PM) and abscisic acid (ABA). The shoot length was found to be inhibited by Cd toxic stress as compared to the control (T1) with an approximate value of 31 cm compared to 44 cm observed under the control condition. However, the shoot length for both poultry manure and ABA treatments were greater at about 52 and 55 cm respectively with the highest treatment being ABA. The T7 (combination of Cd, poultry manure, and ABA) had a shoot length of about 40 cm, which showed partial improvement under Cd stress. The same result was also noted for root length which was reduced from about 10.2 cm in the control to 7.1 cm in Cd stress. The treatment of poultry manure and ABA resulted in root length around 12.1 and 12.7 cm, respectively, while the combination of the two treatments resulted in root length around 9.2 cm. The results showed that Cd toxicity inhibited root growth, but the use of poultry manure and ABA had a positive effect on root growth. Shoot fresh weight was also found to be reduced under Cd stress, from c. 37.5 g (control) to c. 26 g (Cd treated). Using poultry manure and ABA treatments resulted in shoot fresh weights of around 44 and 47 g respectively. The treatment of Cd + poultry manure resulted in about 34 g of shoot fresh weight, indicating a partial recovery from Cd stress. Likewise, under Cd stress the dry weight of the shoots was 2.0 g, and 3.4 and 3.6 g under poultry manure and ABA respectively. The total biomass (shoots dry weight) was about 2.6 g in the combined treatment, suggesting better biomass accumulation under Cd stress. These findings indicated that the amendments might be beneficial for plant growth due to the better uptake of nutrients and performance. The fresh weight of the roots decreased from 2.6 g (control) to 1.8 g (Cd stress). However, the treatments of poultry manure and ABA led to a root fresh weight of about 3.1 g and 3.3 g respectively, while the treatment of both poultry manure and ABA led to an improvement of root fresh weight to about 2.4 g. Similarly, root dry weight under control condition was around 0.55 g while under Cd stress it was 0.38 g. The combined treatment resulted in root dry weight of about 0.50 g while the other two treatments resulted in about 0.65 g and 0.69 g, respectively. The overall results revealed that wheat growth, root growth and biomass accumulation were more adversely influenced by Cd stress than by the use of poultry manure and ABA. When used in combination with Cd stress, they seemed to offer some protection against growth inhibition caused by Cd stress. These changes can affect nutrient availability, water relations and/or physiological responses. However, it is important there is a statistical analysis to see if there are significant differences between treatments.

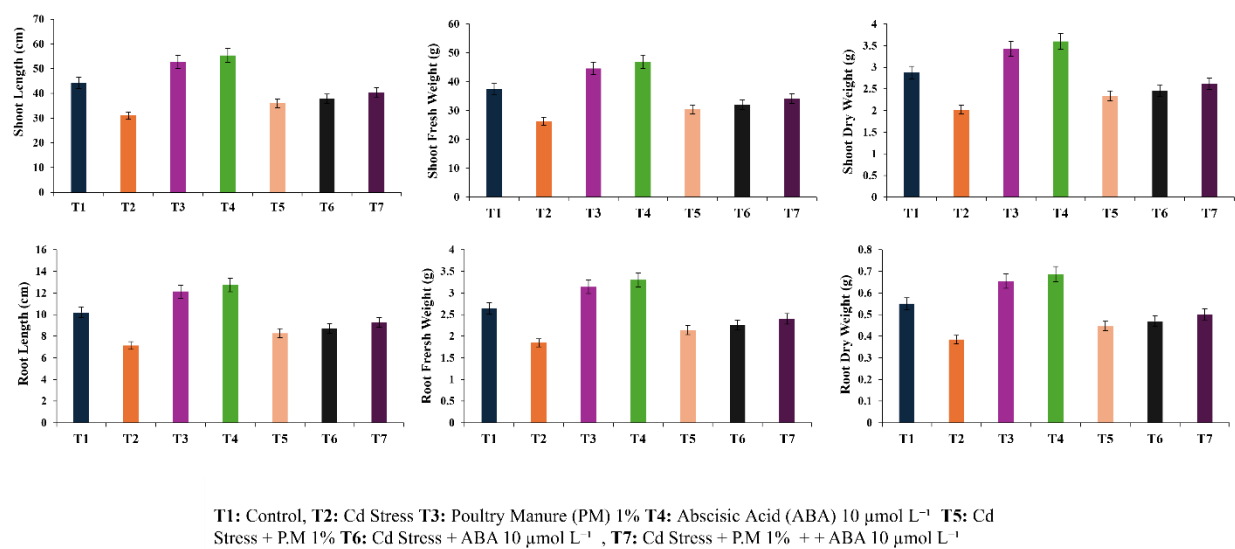
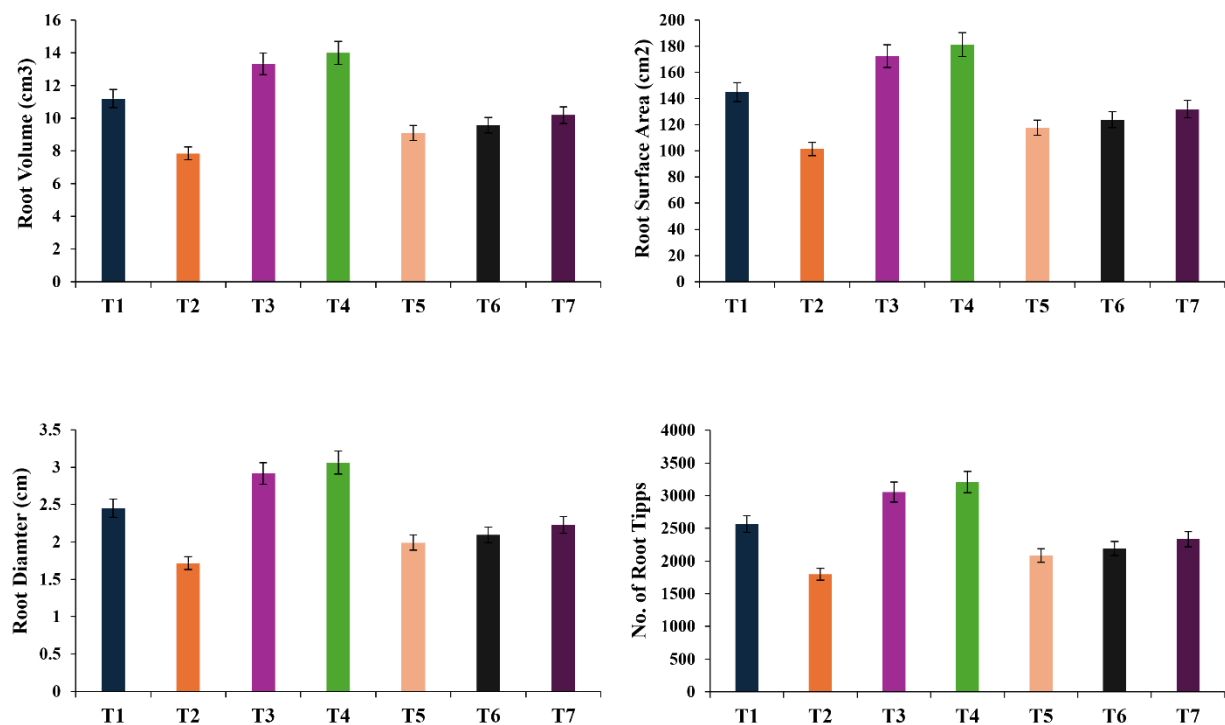


Figure 1. Interactive effect of poultry manure and abscisic acid under cadmium stress on growth attributes of wheat.

3.2. Interactive Effect of Poultry Manure and Absciscic Acid under Cadmium Stress on Root Scan Attributes of Wheat

The results in Figure 2 are for the effects of Cd stress, poultry manure (PM), and abscisic acid (ABA) on root volume, root surface area, root diameter and number of root tips in wheat. Overall, Cd stress caused a negative effect on all root traits evaluated while the individual applications of poultry manure and ABA led to an improvement in root development. However, when the above-mentioned treatments were applied together under Cd stress, some degree of improvement was achieved compared to Cd stress alone. The root volume in T1 (control) was about 11.2 cm³ while it was reduced to 7.8 cm³ under Cd stress (T2). This decrease suggested that Cd stress was limiting root growth and thus the root system volume. The smallest volume of roots was found with the water treatment, while the highest volume of roots was noted for the water treatment containing poultry manure (T3) and the highest volume of roots was observed for the water treatment containing ABA (T4). In the Cd stress, the volume of the roots was about 9.1, 9.6, and 10.2 cm³ in T5, T6, and T7 respectively. The results indicate that the simultaneous application of poultry manure and ABA resulted in more root volume improvement than single application of these amendments under Cd stress. The same trend was seen for the root surface area, which decreased from around 145 cm² in T1 to 100 cm² in T2. Poultry manure and ABA can promote the increase of root surface area to about 172 and 180 cm² respectively. The combined treatment (T7) gave 131 cm² of root surface area under Cd stress while T5 yielded 116 cm² and T6 yielded 123 cm². This growth of root surface area could have led to an improvement in the ability of the wheat plants to search the soil and extract water and nutrients. Root diameter also was smaller in Cd stress (1.7 cm) than in control (2.4 cm). This parameter was raised to around 2.9 and 3.1 cm by poultry manure and ABA, respectively. Root-tip numbers also decreased from about 2,550 to 1,800 with the combined treatments under Cd stress, and about 3,050 and 3,200 with the treatments of poultry manure and ABA, respectively. The joint treatment resulted in a root-tip number around 2350. Overall, Cd stress led to a negative effect on root architecture of wheat, whereas the application of poultry manure and ABA resulted in better root development. It was observed that the combination of Cd and stress had some recovery, possibly due to changes in soil conditions, nutrient availability and regulation mechanisms. However, in order to determine if these differences are statistically significant, it's necessary to analyze the numbers.

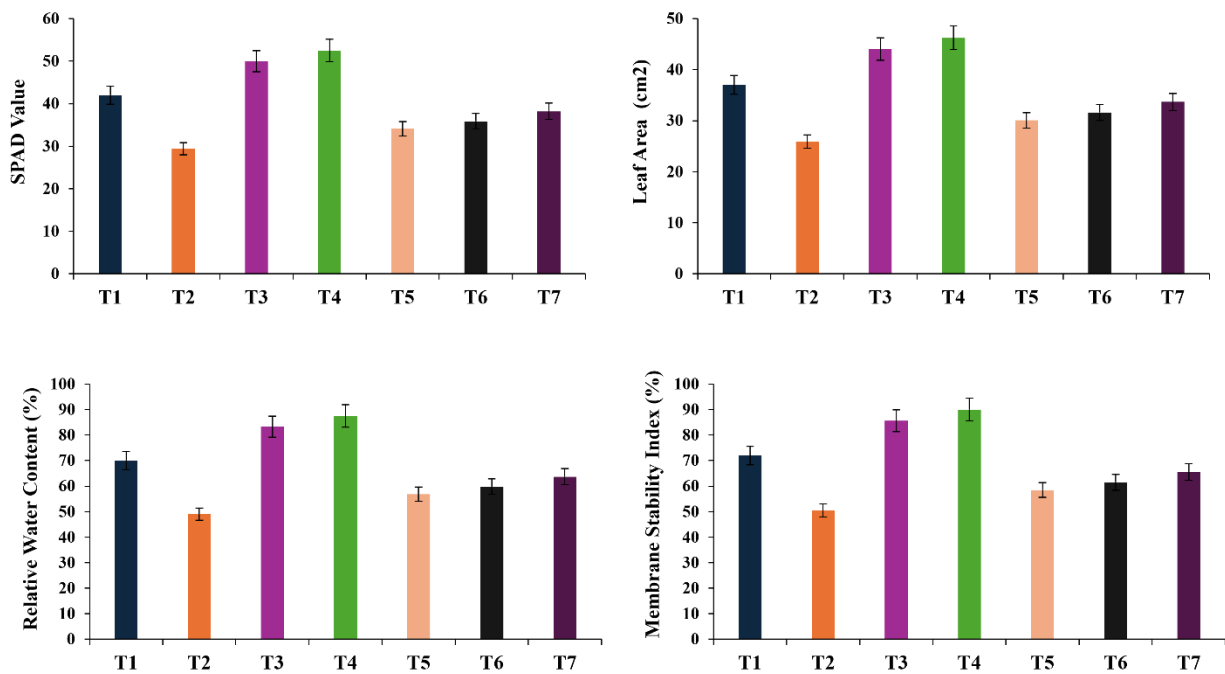


T1: Control, **T2:** Cd Stress **T3:** Poultry Manure (PM) 1% **T4:** Abscisic Acid (ABA) 10 $\mu\text{mol L}^{-1}$ **T5:** Cd Stress + P.M 1% **T6:** Cd Stress + ABA 10 $\mu\text{mol L}^{-1}$ **T7:** Cd Stress + P.M 1% + + ABA 10 $\mu\text{mol L}^{-1}$

Figure 2. Interactive effect of poultry manure and abscisic acid under cadmium stress on root scan attributes of wheat.

3.3. Interactive Effect of Poultry Manure and Abscisic Acid under Cadmium Stress on Physiological Attributes of Wheat

The findings in Figure 3 illustrate the effects of Cadmium (Cd) stress, poultry manure (PM) and abscisic acid (ABA) on the physiological performance of wheat. A set of evaluated parameters were SPAD value, leaf area, relative water content (RWC), and membrane stability index (MSI). All four parameters were reduced under Cd stress, which indicates that the leaf chlorophyll status, leaf development, plant water relations and cellular membrane integrity were adversely affected. However, under Cad stress, poultry manure and ABA enhanced these physiological characteristics and their combination under Cad stress provided partial recovery. From the control treatment (T1), the SPAD value was around 42 to 29 in Cd stress (T2). This decrease indicated that Cd exposure had a negative impact on leaf greenness and may have had an impact on chlorophyll production and/or retention. Poultry manure (T3) raised the value of SPAD to about 50, while ABA (T4) gave the highest value of about 52. In the Cd stress, the poultry manure (T5), ABA (T6) and both T5 and T6 (T7) showed SPAD value of 34, 36 and 38 respectively. Results showed that comprehensive treatment showed more improvement in leaf's chlorophyll status than either amendment in Cd stress. Leaf area was lost from about 37 cm² in T1 to 26 cm² in T2. The average leaf area of the poultry manure was about 44 cm² and that of the ABA was about 46 cm². The area of leaves was improved under combined application under Cd stress as 34 cm², whereas under T5 and T6 30 cm² and 32 cm², respectively, were observed. This leaf area increase may have contributed to increased light interception and photosynthetic potential. Under Cd stress, the relative water content was reduced from around 70% in control to 49%. Poultry manure raised the RWC around 83% and ABA raised it around 87%. T5, T6, and T7 had values of ~57%, ~60%, and ~64% under Cd stress, respectively. Based on the results obtained, it could be concluded that under Cd induced stress, both poultry manure and ABA maintained the water status of the plants. Likewise, the proportion of MSI fell from ~72% in T1 to 50% in T2. MSI was enhanced to about 85% with the addition of poultry manure and 90% with ABA. Combined treatment under Cd stress resulted in an approximate 65% improvement in MSI, which meant a partial recovery of membrane stability. Poultry manure and ABA had beneficial effects on wheat physiological performance, while Cd stress had detrimental effects on all of the above. The two applications together yielded some beneficial effect, but statistical analysis will be needed to determine the significance of any differences in the effects of the treatments.

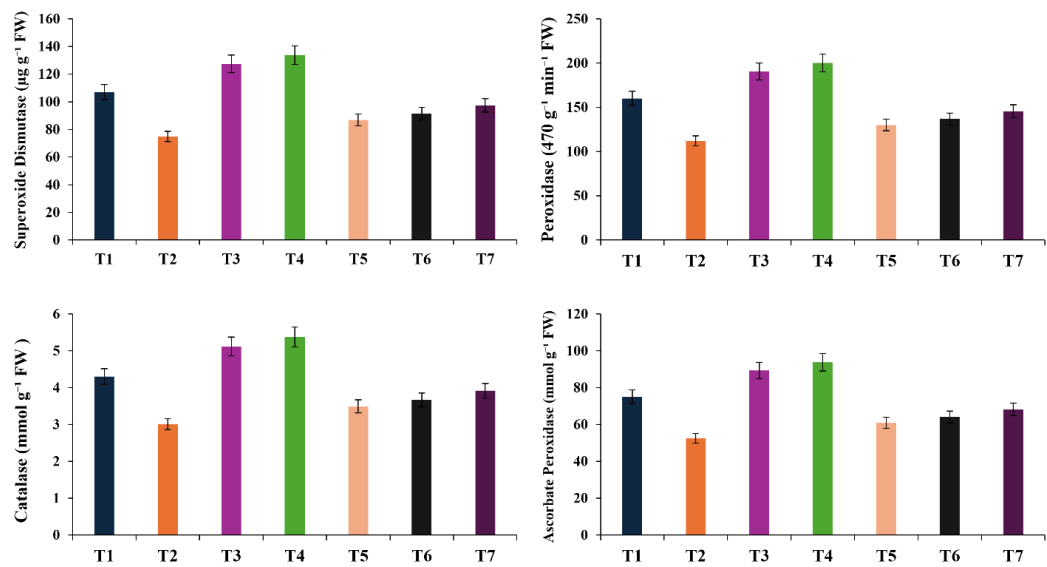


T1: Control, T2: Cd Stress T3: Poultry Manure (PM) 1% T4: Absciscic Acid (ABA) 10 $\mu\text{mol L}^{-1}$ T5: Cd Stress + P.M 1% T6: Cd Stress + ABA 10 $\mu\text{mol L}^{-1}$ T7: Cd Stress + P.M 1% + + ABA 10 $\mu\text{mol L}^{-1}$

Figure 3. Interactive effect of poultry manure and abscisic acid under cadmium stress on physiological attributes of wheat.

3.4. Interactive Effect of Poultry Mamure and Absciscic Acid under Cadmium Stress on Antioxidant Enzyme Activities of Wheat

The results presented in Figure 4 show the effects of the cadmium (Cd) stress, poultry manure (PM) and abscisic acid (ABA) on the antioxidant enzyme activities in wheat. Superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) were the enzymes. The enzymes play a significant role in the plant antioxidant defense network as they help regulate the production of ROS and prevent damage to cellular structures. Results showed that Cd stress caused a decrease in activities of all four enzymes while treatments with poultry manure and ABA showed an increase in the activities of these enzymes. The activity of SOD under Cd stress (T2) declined from about 107 $\mu\text{g g}^{-1}$ fresh weight in the control treatment (T1). Poultry manure (T3) had a SOD activity of about 127 $\mu\text{g g}^{-1}$ fresh weight and ABA (T4) had the highest value of about 134 $\mu\text{g g}^{-1}$ fresh weight. The values for poultry manure (T5), ABA (T6), and combination of both (T7) were about 87, 91, and 97 $\mu\text{g g}^{-1}$ fresh weight under Cd stress, respectively. The two treatments together showed an increase in SOD activity compared to either amendment alone under Cd stress, which suggested an additive effect. The activity of POD also remained similar with a decrease from 160 to 112 $\mu\text{mol g}^{-1} \text{min}^{-1}$ fresh weight from T1 to T2. Poultry manure and ABA stimulated POD activity to ~190 and 200 $\mu\text{mol g}^{-1} \text{min}^{-1}$ fresh weight respectively. The combination of the two treatments resulted in a value of Cd stress of about 145 $\mu\text{mol g}^{-1} \text{min}^{-1}$ fresh weight, higher than T5 and T6. Under Cd stress, the CAT activity of the control decreased to 3.0 mmol g fresh weight. Poultry manure and ABA had the highest CAT activity, approximately 5.1 and 5.4 mmol g^{-1} fresh weight, respectively. The treatment of Cd stress resulted in an increase in the CAT activity to about 3.9 mmol g^{-1} FW under combined treatment. Similarly, the levels of APX activity decreased from about 75 nmol g^{-1} fresh weight at T1 to 52 nmol g^{-1} fresh weight at T2. The activity of APX was enhanced by the use of poultry manure and ABA with values of around 89 and 94 nmol g^{-1} fresh weight respectively, and T7 with around 69 nmol g^{-1} fresh weight. In general, the results indicated that Cd stress caused a decrease in the activity of antioxidant enzymes, while poultry manure and ABA enhanced the wheat antioxidant enzyme activity. Enzymatic activity was higher when they were used together under Cd stress than either one was used alone under Cd stress. Such responses could be related to increased antioxidants protection against Cd-induced oxidative stress. The observed trends should, however, be corroborated by a statistical analysis before stating that there was a significant difference between treatments.

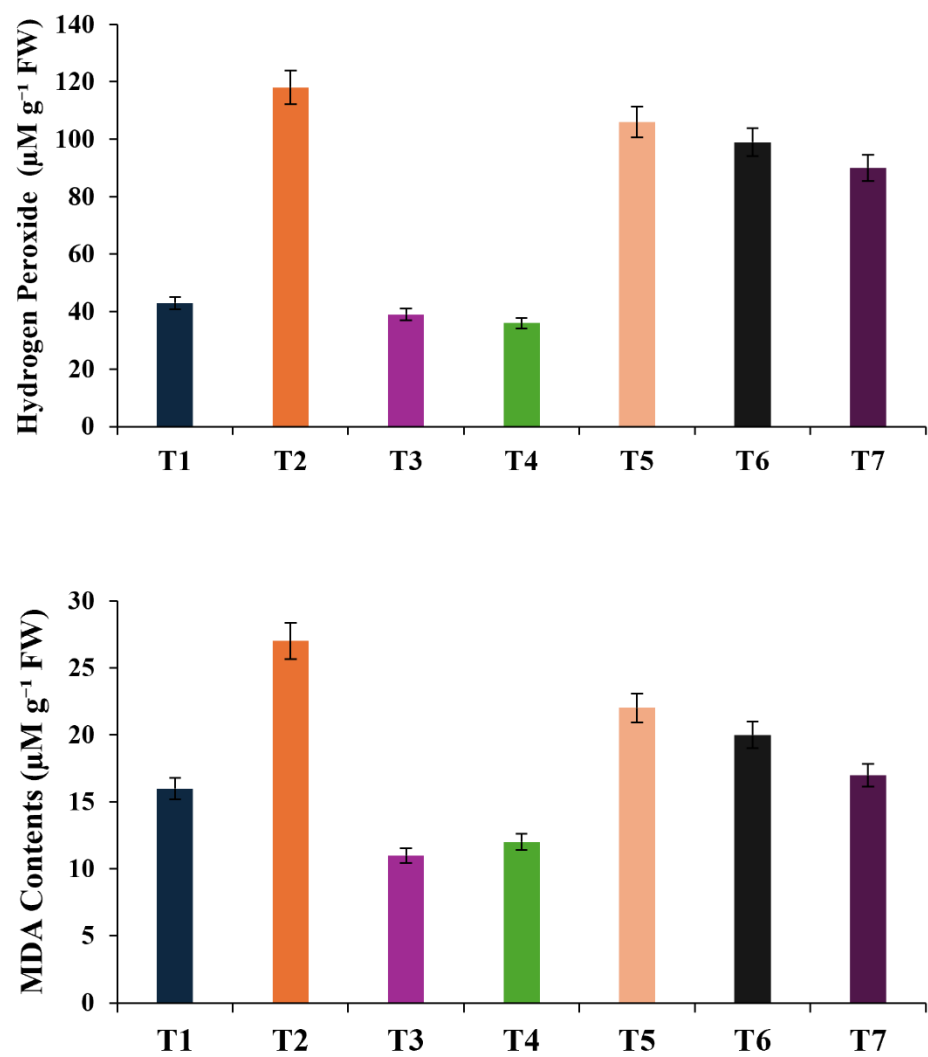


T1: Control, T2: Cd Stress T3: Poultry Manure (PM) 1% T4: Abscisic Acid (ABA) 10 $\mu\text{mol L}^{-1}$ T5: Cd Stress + P.M 1% T6: Cd Stress + ABA 10 $\mu\text{mol L}^{-1}$ T7: Cd Stress + P.M 1% + ABA 10 $\mu\text{mol L}^{-1}$

Figure 4. Interactive effect of poultry manure and abscisic acid under cadmium stress on antioxidant enzyme activities of wheat.

3.5. Interactive Effect of Poultry Manure and Abscisic Acid under Cadmium Stress on Oxidative Stress Indicator of Wheat

The cadmium (Cd) stress induced the changes in hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) content in wheat which have been presented in Figure 5 in the presence of poultry manure (PM) and abscisic acid (ABA). Both are relevant parameters for plants as a measure of oxidative damage and stress. Hydrogen peroxide is a reactive oxygen species which could be accumulated under environmental stress, while MDA is widely utilized as a marker of lipid peroxidation and damage to cell membranes. Under Cd stress, both contents of H_2O_2 and MDA were significantly elevated, while both poultry manure and ABA treatments led to decreased H_2O_2 and MDA accumulation. The hydrogen peroxide content was around 42 $\mu\text{M g}^{-1}$ fresh weight in the control treatment (T1) while Cd stress alone (T2) increased it to about 118 $\mu\text{M g}^{-1}$ fresh weight. This large increment suggested an enhancement of the oxidative stress in wheat plants due to Cd exposure. However, poultry manure alone (T3) lowered the level of H_2O_2 to about 39 $\mu\text{M g}^{-1}$ fresh weight and ABA alone (T4) further lowered H_2O_2 to about 36 $\mu\text{M g}^{-1}$ fresh weight. However, when the Cd stress was brought into the picture and poultry manure (T5) was applied, the H_2O_2 level was reduced to ~106 $\mu\text{M g}^{-1}$ fresh weight. In the same way, it was lowered to ~99 $\mu\text{M g}^{-1}$ fresh weight with ABA application under Cd stress (T6). The synergistic effects of poultry manure and ABA under Cd stress (T7) further lowered the Cd concentration to ~ 90 $\mu\text{M g}^{-1}$ fresh weight. The overall results indicated that the combined treatment was more effective than either of the treatments alone in protecting against Cd-induced oxidative stress. The same trend was noted with regard to MDA content. The stress caused by Cd caused MDA content to be increased to about 27 $\mu\text{M g}^{-1}$ fresh weight while the control treatment had about 16 $\mu\text{M g}^{-1}$ fresh weight. The amount of MDA content was decreased to around 11 and 12 $\mu\text{M g}^{-1}$ fresh weight when poultry manure and ABA were applied separately. Cd stress decreased the content of MDA to about 22 $\mu\text{M g}^{-1}$ fresh weight due to the application of poultry manure and further reduced by about 20 $\mu\text{M g}^{-1}$ fresh weight when ABA was applied. The use of poultry manure and ABA together reduced the content of MDA further to about 17 $\mu\text{M g}^{-1}$ fresh weight which was close to the control level. Generally, results showed that Cd stress caused oxidative damage of wheat, while the application of poultry manure and ABA reduced the oxidative damage. The application of both appeared to be most effective for the Cd-stressed treatments, possibly because of greater antioxidant defense and/or physiological protection. There needs to be statistical analysis to determine if the observed differences were significant.



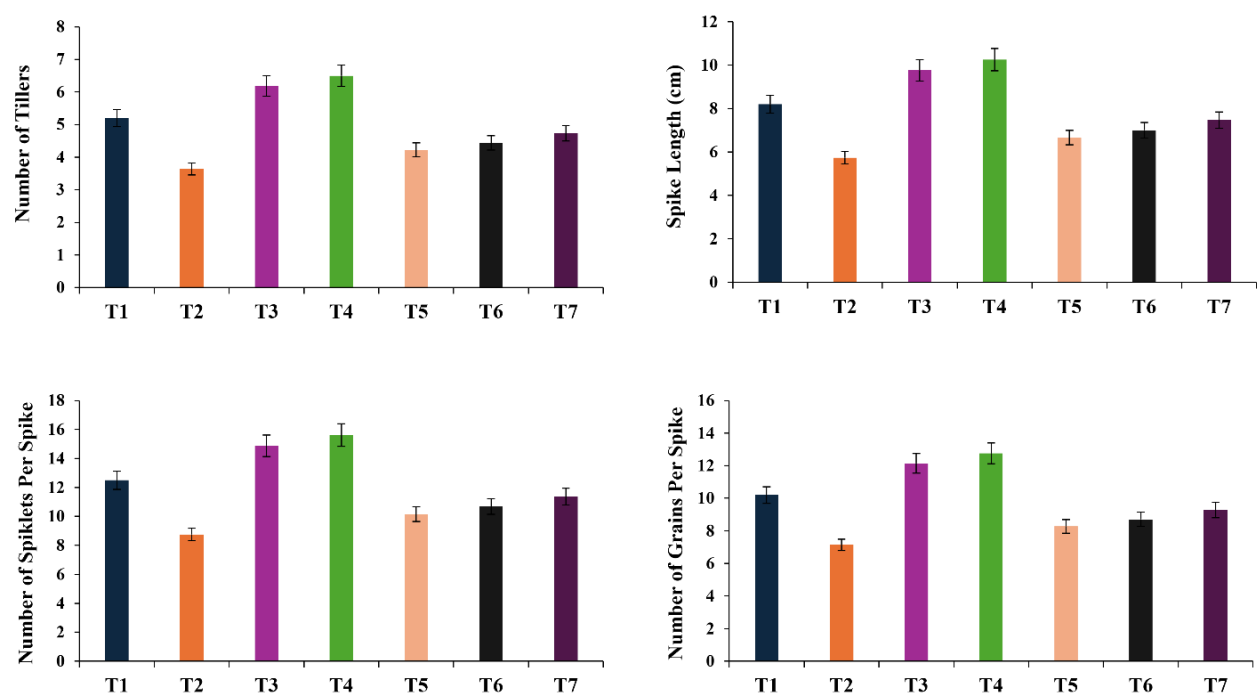
T1: Control, **T2:** Cd Stress **T3:** Poultry Manure (PM) 1%
T4: Absciscic Acid (ABA) 10 µmol L⁻¹ **T5:** Cd Stress +
P.M 1% **T6:** Cd Stress + ABA 10 µmol L⁻¹ **T7:** Cd Stress +
P.M 1% + + ABA 10 µmol L⁻¹

Figure 5. Interactive effect of poultry manure and abscisic acid under cadmium stress on oxidative stress indicator of wheat.

3.6. Interactive Effect of Poultry Manure and Absciscic Acid under Cadmium Stress on Yield-Related Attributes of Wheat

Results of Cd stress on poultry manure (PM) and abscisic acid (ABA) in wheat are presented in Figure 6, which shows the effects on the important yield-related attributes of wheat such as number of tillers, spike length, number of spikelets per spike and number of grains per spike. The parameters are key indicators of wheat reproductive development and yield potential. It was found that Cd stress decreased all the measured parameters related to yield while the application of poultry manure and ABA alone and in combination with Cd stress improved these attributes. Cd stress alone (T2) reduced the number of tillers from about 5.2 in control (T1) suggesting Cd toxicity has a negative effect on tiller formation. Increasing the number of tillers was observed, with the highest value about 6.5, for ABA (T4), and the lowest value about 6.2 for poultry manure (T3). The number of tillers under Cd stress increased significantly to about 4.2, 4.4 and 4.7 tillers under poultry manure (T5), ABA (T6) and their mixture (T7) respectively. This answer indicated that the joint treatment was more effective in improving the tiller production compared with the individual treatments with the amendments under Cd stress. Similarly, the length of spike also reduced from about 8.2 cm in T1 to 5.7 cm in T2. The application of both poultry manure and ABA led to a stretch in spike length of about 9.8 and 10.3 cm. The combined treatment (T7) led to an increase in spike length to about 7.5 cm, whereas the stress alone (T5 and T6) resulted in spike length of 6.6 and 7.0 cm respectively. The results showed that Cd induced stress was effectively managed by restricting spike development with the application of poultry manure and ABA. Under Cd stress, the number of spikelets per spike was reduced from about 12.5 (control) to 8.7. This parameter was significantly raised by the use of poultry manure to about 14.8, while the use of ABA resulted in about 15.6 spikelets per spike. A. Cd stress produced about 10.1, 10.6 and 11.3 spikelets per spike in T5, T6 and T7, respectively. Likewise, the number of grains per spike decreased from about 10.2 in T1 to 7.1 in T2. Under Cd stress, the combined result was improved to about 9.3 grains per spike; the other treatments were about 12.1 and 12.7 grains per spike with poultry manure and ABA, respectively. The results overall showed that Cd stress negatively influenced wheat tillering and spike development, thus limiting yield potential. Under Cd stress, poultry manure and ABA enhanced the measured attributes, and the combination of both were more effective for recovery than either supplement alone. These changes can relate to increased

nutrient availability, decreased oxidative damage and physiological function. But it would be necessary to obtain grain yield per plant and to statistically validate the overall yield response, and differences between treatments.



T1: Control, **T2:** Cd Stress **T3:** Poultry Manure (PM) 1% **T4:** Absciscic Acid (ABA) 10 $\mu\text{mol L}^{-1}$ **T5:** Cd Stress + P.M 1% **T6:** Cd Stress + ABA 10 $\mu\text{mol L}^{-1}$ **T7:** Cd Stress + P.M 1% + ABA 10 $\mu\text{mol L}^{-1}$

Figure 6. Interactive effect of poultry manure and abscisic acid under cadmium stress on yield-related attributes of wheat.

3.7. Discussion

Wheat under Cadmium (Cd) stress showed increased levels of oxidative stress indicators along with decreased spike length, spikelet number per spike, tiller number and grain number per spike. These results indicated that Cd toxicity affected the normal physiological function and reproductive development, which is in line with the previous report that Cd accumulation decreased wheat growth, nutrient uptake and yield potential (Halim et al., 2021). The higher level of hydrogen peroxide (H_2O_2) in the Cd stressed wheat suggests that Cd stress stimulated the production of reactive oxygen species (ROS) more than the plant's regulatory systems. Too much ROS may impair plant growth and physiological processes, including plant metabolism, by attacking cell membranes and other components of the cellular structure (Gao et al., 2021). The increased malondialdehyde (MDA) levels found under Cd stress further confirm the occurrence of oxidative injury, since MDA levels serve as a marker for membrane lipid peroxidation. Hussain et al. (2022) reported similar increases in the production of H_2O_2 and MDA under Cd stress and improvements following protective treatments in wheat grown with boron and biochar. The decrease in oxidative-stress markers after poultry manure application could be linked to better soil fertility, organic matter content, and the possibility of changing the Cd availability in the root zone. Shaghaleh et al. (2024) found that organic amendments such as poultry manure, farm manure, rice-husk biochar, affected the performance of the cereals and the uptake of Cd in wheat and rice in a three-year field study. The positive reaction following ABA treatment could be associated with its role in the regulation of physiological changes associated with environmental stress.

Lin et al. (2025) demonstrated that wheat growth and root morphology were enhanced, soil-available Cd concentration was reduced and the accumulation of Cd in plant tissues was decreased by adding biochar along with ABA to soil. In the present study, the respective treatments such as Cd, PM and ABA have demonstrated that combined treatment of Cd + PM + ABA exhibited better effectiveness in reducing oxidative stress, indicating that soil amendment + oxidative stress regulation is also complementary. The same beneficial effects of organic amendments on Cd bioavailability and wheat performance were reported by Rahim et al. (2024) when they compared wheat-straw biochar with chicken-waste compost in Cd-contaminated soil. The number of tillers decreased with Cd exposure, suggesting that the production of productive shoots was limited by Cd toxicity, and thereby shortening the number of spikes that could contribute to final grain yield. The findings of Nawaz et al. (2024) confirmed that Cd stress had an inhibitory effect on wheat growth and yield related traits, and nitric oxide had a stimulatory effect on tiller number and other growth traits. The higher number of spikelets and the longer spikes in Cd stress condition indicate the adverse growing conditions due to Cd stress affected reproductive development. The same results were obtained by Hussain et al. (2022) who reported that Cd toxicity caused a decrease in wheat yield and yield associated parameters while boron and biochar enhanced several physiological and agronomic parameters. The reduced grains per spike suggests that Cd-associated injury not only affected vegetative growth, but also a significant aspect of reproductive performance. The wheat yield attributes also showed improvements with nitric oxide application under Cd stress, as reported by Nawaz et al. (2024) indicating that any mitigation that lessens the stress can help sustain wheat productivity. This decrease in oxidative damage after protective treatments could also be correlated with the alteration in the activity of antioxidant enzymes such as ascorbate peroxidase, peroxidase, catalase and superoxide dismutase. In wheat plant under Cd stress, Wu and Lu (2024) found that selenium application increased the antioxidant ability, the photosynthetic performance and

decreased the accumulation of H₂O₂ and MDA. The same protective effect has been previously reported for Methyl jasmonate, which may modify antioxidant defense and Cd distribution in wheat tissues.

However, Zhang et al. (2024) reported that methyl jasmonate not only enhanced the stress response of the wheat but also decreased the transport and accumulation of Cd in the grain, revealing a concern for crop safety as well as growth. The results also support the research conducted on other plant growth regulators and protective chemicals to mitigate Cd toxicity to wheat. Zulfiqar et al. (2024) showed that wheat varieties showed varying responses of several growth and biochemical parameters under the influence of salicylic acid concentrations and cultivars under Cd stress. The treatment effect of combined poultry manure and ABA must be treated with great caution, as the poultry manure/ABA combination did not necessarily show better treatment effect than only Cd. A good example of integrated research for biochar and soil-amendment combined with plant-regulators is presented by Haider et al. (2025) which showed that the application of biochar and 24-epibrassinolide enhanced wheat resilience to Cd and drought stress. Present study indicates that the use of poultry manure and ABA as complementary treatments are effective in enhancing wheat performance under Cd stress, especially reducing the indicators of oxidative stress and maintaining yield-related characteristics. Next, soil, root, shoot and collected grain samples should be analyzed for Cd levels in future field trials to assess the reduction of food-chain risk associated with the observed growth improvement, as highlighted by Sehrish et al. (2024). Additionally, the results show that the assessment of cadmium tolerance should consider an integrated approach, such as individual evaluation of oxidative stress, vegetative growth and reproductive performance, instead of a single parameter. The decrease in H₂O₂ and MDA, along with the increase in the number of tillers, spike length, spikelets per spike and grains per spike in the plants treated with PM and ABA indicate that the preservation of the cellular protection might be a factor in improved reproductive development under Cd stress (El-Sappah et al., 2023). But this association must be confirmed with measures of antioxidant enzyme activity, photosynthetic activity, nutrient uptake and final grain production as well as statistical comparisons between treatments. Lin et al. (2025) found that biochar along with ABA promoted activities of antioxidant defense and Cd uptake was reduced in wheat, and Shaghaleh et al. (2024) reported that the use of organic amendments can affect the growth of crops and Cd uptake under field conditions. Future studies should compare these two treatments with different soil types, wheat cultivars, and application rates, to see if the treatments are consistent and to assess if reduced Cd levels in the harvested grain are associated with any improvement in crop performance.

4. Conclusion

The present study showed that Cadmium stress had negative effects on wheat growth and reproductive performance, whereas it was found to be increasing the Cadmium stress indicators related to oxidative stress. The higher level of H₂O₂ and MDA indicated increased oxidative pressure and lipid peroxidation of the membranes in Cd exposed plants. Cadmium stress also led to a decrease in the number of tillers, spike length, spikelet per spike and grains per spike, which suggested negative impacts on important yield components. These parameters were better when combined with application of poultry manure and abscisic acid than in Cd stress alone. The overall treatment promisingly exhibited a reduction in oxidative damage and partially maintained the reproductive development under Cd stress. The results indicate that there is a potential for using organic soil amendments and plant growth regulators as supplementary measures to enhance wheat tolerance under heavy metal stress. However, the efficacy of their Cd reducing ability and grain yield need to be evaluated. Validation and assessment of grain Cd concentration at the field scale are crucial before these treatments can be recommended for the safe and sustainable production of wheat in contaminated soils.

5. Recommendations

There is a need to conduct further experiments to test the different application rates and growth stages of poultry manure and abscisic acid in order to determine the most effective treatment combination. To ensure consistency of the wheat cultivar and soil type responses, field trials are recommended. Future studies should include the activities of antioxidant enzymes, photosynthetic performance, nutrient uptake, soil available Cd and root, shoot and grain accumulation. Final grain yield and grain quality should also be evaluated to assess agronomic and food safety advantages of these treatments. Under the cadmium stress, statistical interaction analysis is needed to determine if combined applications are more effective than individual applications.

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