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Exploring the Potential of Moringa Leaf Extract for Mitigation of Cadmium Stress in *Triticum aestivum* L.

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Abstract: Cadmium (Cd) is one of the most toxic metals accumulated in wheat grains. Daily intake of Cd through food is posing serious health problems. There is an urgent need to reduce the uptake and accumulation of Cd in wheat and other cereal crops. In this study, we investigated the potential of moringa leaf extract (MLE) in decreasing the Cd toxic effects in wheat (*Triticum aestivum* L.) cv. A.S. 2002. Sowing was carried out in pots under shade and natural light conditions. Two Cd concentrations (500 µM and 1000 µM) were applied with and without MLE (0 and 3%). Results revealed that plant growth parameters viz diameter of stem, number of leaves, leaf area, photosynthetic pigments were improved with MLE application under Cd stress. Moreover, biochemical attributes and osmolytes such as total soluble sugars, and soluble proteins, phenolic content and flavonoids were negatively effected by Cd stress and were improved under MLE treatment. Moreover, Cd stress enhanced phytotoxicity as higher ROS accumulation is observed under both stress conditions, whereas after MLE application MDA and H_2O_2 accumulation was significantly decreased. Based on current observations, MLE was effective in mitigating the biological and toxic effects of Cd by promoting the growth attributes and mineral contents in wheat. Further experiments are needed for discovering the molecular mechanisms underlying MLE and host plant interactions involved in Cd stress mitigation.

Keywords: cadmium; wheat; antioxidant; morphological; biochemical; light; oxidative stress

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1. Introduction

Wheat (*Triticum aestivum*) is the most leading cereal crops and major food source for human beings. Unfortunately, agricultural products leads to the increased the risks of the contamination of wheat particularly cadmium (Cd), leads to increase the risk of many inflammatory, metabolic and chronic diseases [1]. Contamination of wheat occurs through anthropogenic and natural sources that are major contributors to decreasing overall yield production [2]. Anthropogenic sources mainly included phosphate fertilizers, industrial emissions, and mining activities that increased the risks of accumulation of cadmium in wheat. Industrial effluents are also a major source of cadmium that enters the freshwater used for the cultivation of wheat and cereal crops including *Triticum aestivum* L., *Oryza sativa* L. and *Zea mays* L [3,4].

Wheat has high Cd absorption capacity as compared to other cereal crops. Accumulation of cadmium in different parts of wheat appears to be toxic [5]. Recent studies showed

that uptake of cadmium in wheat occurs by roots and is then translocated into the aerial parts. Translocation of cadmium appears to be a fast process where high concentration of cadmium inhibits the growth in aerial parts and also interferes with metabolic processes like photosynthesis, respiration, transpiration, chlorophyll biosynthesis and stomatal conductance [6].

Currently, natural biostimulants are used to overcome the metal toxicity that improves the yield under stress conditions [7]. Biostimulants are rich source of diverse compounds, plant growth promoting substances with positive effects on plants. These compounds regulated the plant activities, diminished oxidative stress and promoted plant growth and developmental stages [8,9]. Moringa leaf extract (MLE) is one of the most natural biostimulant primarily containing higher nutrient contents that help the plant to improve growth and inhibit the cadmium phytotoxicity in many crops such as *Triticum aestivum* L., *Phaseolus vulgaris* L. and *Lepidium sativum* L. [10]. According to numerous investigations, MLE is also a rich source of antioxidants, amino acids, riboflavin, ascorbic acid folic acid, pyridoxine, vitamins such as A, B and C, which are essential for the control of physiological, metabolic, enhanced development, yield attributes and zeatin that regulate the physiological and biochemical processes and improve the yield under harsh environmental conditions [11,12].

MLE is also used for the absorption of toxic metals such as cadmium in soils due to the presence of thiol-containing proteins that biochemically interact hydroxyl group of cadmium. It has been reported that the application of MLE improved the antioxidant enzyme system of wheat. It also reduced the oxidative stress from free radicals species that occurred under high concentrations of cadmium [13].

Fluctuations of plant growing conditions such as temperature, light, humidity, nut rient availability etc. during growing season can have unpredictable effects on plant yield. Such as, changes in solar radiation is one of the factors controlling grain yield. Increased light intensity stimulates leaf production, and it enhances the number of leaves and shoots of plants under light. The intensity of light affects the plant characteristics. Plants grown under shade are more susceptible to photoinhibition, while on the other hand, low light may also result in an over excitation of electron transport systems, potentially resulting (ROS) that promotes oxidative stress, such as hydrogen peroxide, superoxide, and singlet oxygen. The excess of these ROS affects the production of macromolecules, disrupts the antioxidant activities, and decreases plant height, root length, soluble proteins and antioxidant enzyme activities [6,14].

Keeping in view literature reported, there is a lack of necessary information for revealing the potential of MLE for decreasing the Cd tolerance in wheat. To the best of our knowledge, this type of approach with MLE soil application was not reported in the literature before. Therefore, the current study was designed to explore the potential of MLE on Cd tolerance in A.S. 2002 *Triticum aestivum* L. In addition, we explore the morphological parameters, biomass accumulation, antioxidants, osmolytes and mineral constituents under two light conditions of wheat plant with and without MLE application under Cd stress.

2. Material and Method

2.1. Study Site and Experimental Design

This study was conducted at the old botanical garden of University of Agriculture Faisalabad. Wheat (*Triticum aestivum* L.) cv. A.S 2002 was used for pot experiment, conducted under two light conditions:

Cadmium chloride was used to prepare 500 micromolar and 1000 micromolar cadmium solutions. Extract of moringa leaf was prepared by crushing the fresh moringa leaves before using them for experiment [15]. Six treatments were applied in CRD design and were tagged as Control (MLE0%, Cd0%), MLE3%, Cd500%, Cd500+MLE3%, Cd1000, Cd1000+MLE3%). Each of six treatments were in triplicate with two light conditions: direct sunlight and in the shade. MLE solution was applied with while watering the plant with water.

2.2. Harvesting and Data Collection

Cadmium stress was applied one month after sowing and plants were harvested after twenty days of stress application. All plant samples were washed with distilled water. Measuring tape was used for the measurement of the fresh plant shoot and the root lengths (cm). After harvest, the roots and shoots fresh weights (g/plant) were immediately noted down by using digital weighing balance. Diameter of shoots (mm), leaf area (cm^2), number of tillers (per plant) and number of leaves (per plant) were also noted. Samples then finally oven dried at 65 °C for 72 h and then were used for the analysis of ion and measuring dry weight.

2.3. Determination of Photosynthetic Pigments

Chlorophyll a, b and carotenoid contents (mg/g FW) were measured by using the method of Arnon et al. [16]. 0.1 g of the fresh plant was grinded by adding the 10 mL of 80% acetone, and absorbance was recorded at 663, 645 and 480 nm by using the spectrophotometer. Respective formulas were applied for the determination of the chlorophyll a, b and carotenoid from their obtained OD values [16].

2.4. Determination of Anthocyanin (µg/g FW)

Anthocynanin content was calculated by following the method of Stark et al. [17]. Fresh leaf material (0.1 g) was homogenized into the1 mL of acidic methanol (1% HCL v/v), and reaction mixture was heated up at 50 °C for one hour. Absorbance was recorded at 535 nm by using a spectrophotometer [17].

2.5. Determination of Total Phenolic Contents (µg/g FW)

Phenolic contents were evaluated by using method adopted by the Julkunen-Tiitto [18]. Fresh plant samples of known weight were homogenized with 80% acetone. After centrifugation at 1000× g for 15 min, supernatant (0.1 mL), water (2 mL) and Folin-Ciocalteau's phenol reagent (1 mL) were mixed well in falcon tube. Thereafter, 20% Na_2CO_3 (2 mL) was added along with distilled water (10 mL). Absorbance was recorded at 750 nm by using the spectrophotometer.

2.6. Determination of Flavonoid (µg/g FW)

Flavonoid content was measured by using the method reported by Mukherjee et al. [19]. Fresh plant material of known plant weight was homogenized in 80% acetone. After that, 4 mL DW added into the 1 mL of extracted material. After five minutes, 5% $NaNO_3$ and 10% $AlCl_3$ were added to the samples followed by addition of 1M NaOH after one minute. Thereafter, absorbance was checked at 510 nm by using the spectrophotometer [19].

2.7. Determination of Total Soluble Sugars (µmol/g FW)

Total soluble sugar (TSS) contents were measured by the method of Yoshida et al. [20]. Fresh plant material (0.1g) was boiled into the 5 mL distilled water and then filtrated by using the filter paper and diluted up to 10 mL. Anthrone reagent (5 ML) was added to the reaction mixture and heated at 90 °C for 20 min. Finally, absorbance was observed at 620 nm [20].

2.8. Determination of Malondialdehyde (MDA) (µmol/g FW)

MDA values were determined by using the method of Heath & Packer [21]. Briefly, leaf samples of known weight were grinded into 0.1% TCA (Trichloroacetic Acid) and centrifuged them at 12,000 rpm for 15 min. Thereafter, 1mL of supernatant was mixed with 2 mL of 0.5% TBA in 20% TCA and the mixture was incubated at 95 °C. After 30 min of incubation reaction was stopped by immediately placing in an ice bath. Finally, after centrifugation (12,000 rpm, 10 min, 4 °C) absorbance was measured at 600 nm and 532 nm [21].

2.9. Determination of Hydrogen Peroxide (H_2O_2) (µmol/g FWT)

Concentration of H_2O_2 was determined by following the method adopted by Velikova et al. [22]. Leaf samples of known weights were grinded into 5 mL of 0.1% TCA and centrifuged at 12,000 rpm for 15 min. In 1 mL of supernatant, 0.5 mL of 10 mM potassium phosphate buffer was added followed by addition of 1 mL of potassium iodide. Finallay, the absorbance was measured at 39 nm by using the spectrophotometer [22].

2.10. Determination of Calcium (Ca) and Sodium (Na) and Potassium (K) (mg/g DW)

Plant samples were dried at 65 °C in an oven and then each sample was grounded into powder form. For 0.1 g of powdered sample, 6 mL of HNO_3 was added to each flask. Flasks were kept on hot plate and 2–3 drops of hydrogen peroxide were added to each flask. After cooling down filter paper was used to filter the mixture and distilled water was added to raise the volume til 50 ML [23].

Concentrations of calcium, sodium and potassium were determined by following the method of Yoshida et al. [20]. Concentrations of Ca^{2+}, Na^{2+} and K^{+} were estimated by using the flame photometer (Sherwood model, 410 UK).

2.11. Statistical Analysis

ANOVA (analysis of variance) was done in our study to analyze the data under the CRD (Completely Randomized Design). Statistix 8.1 was used to analyze the physiological and morphological data. The LSD (least significant difference) test was performed to compare the treatment means with a probability level of 5.0 percent.

3. Results

3.1. Effect of MLE on Morphological Attributes of Plants

3.1.1. Shoot Length

Figure 1A shows the effect of MLE application on the shoot length of wheat facing Cd stress. Results revealed that the application of 3% MLE along with combinations of 500 µM and 1000 µM (under light) cadmium solutions increased the shoot length by 17.28% and 18.72% compared to control. This increase was less under shade compared to light but was still higher under Cd 500+MLE (13.7%) and Cd1000+MLE (16.3%) compared to their control under shade. More interestingly, 3% MLE with both cadmium treatments even resulted in longer shoot lengths compared to MLE 3% alone treatment (higher in light compared to shade).

3.1.2. Root Length

Figure 1B shows the effect of MLE application on the root length of wheat facing Cd stress. Results revealed that the application of 3% MLE along with combinations of 500 µM and 1000 µM (under light) cadmium solutions increased the root length by 17.67% and 33.20% compared to control. This increase was less under shade compared to light but was still higher Cd1000+MLE (10.73%) compared to their control under shade. More interestingly, 3% MLE with both cadmium treatments even resulted in longer root lengths compared to MLE 3% alone treatment under light.

3.1.3. Leaf Area

Figure 1C shows the effect of MLE application on leaf area of wheat facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) significantly reduced the leaf area by 31.3% and 49.83% as compare to control, while, on the other hand, application of 3% MLE along with combinations of 500 µM and 1000 µM cadmium solutions increased the leaf area of wheat by 4.90% and 6.36 compared to respective Cd stressed conditions.

Moreover, both Cd treatments with 3% MLE also showed reduction in leaf area compared to MLE 3% treatment under both light and shade conditions.

3.1.4. Number of Leaves

Figure 1D shows the effect of MLE application on number of leaves of wheat facing Cd stress. Results revealed that the application of 3% MLE along with combinations of 500 µM and 1000 µM cadmium solutions (under light) didn't significantly increased the number of leaves as compared to control. Only increase was observed under Cd 500 µM + MLE 3% compared to Cd 500 µM under light.

Number of leaves were higher under both light and shade conditions in MLE3% compared to both CD treatments with MLE.

3.1.5. Number of Tillers

Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) significantly reduced the number of tillers by 20% and 50% as compare to the control, while, on the other hand, application of 3% MLE along with combinations of 500 µM and 1000 µM cadmium solutions increased the number of tillers of wheat by 25% each compared to control (Figure 1E).

3% MLE with both cadmium treatments also resulted in higher tillers under light conditions compared to 3%MLE alone.

3.1.6. Stem Diameter

Figure 1F shows the effect of MLE application on stem diameter of wheat facing Cd stress. Results revealed that the under light, application of 3% MLE along with combinations of 500 µM cadmium solutions increased the stem diameter by 4.44% as compared to control. This increase was high under shade compared to light Cd 500+MLE (24.4%). 3% MLE increased stem diameter under light and shade significantly compared to cadmium treatments+MLE3%.

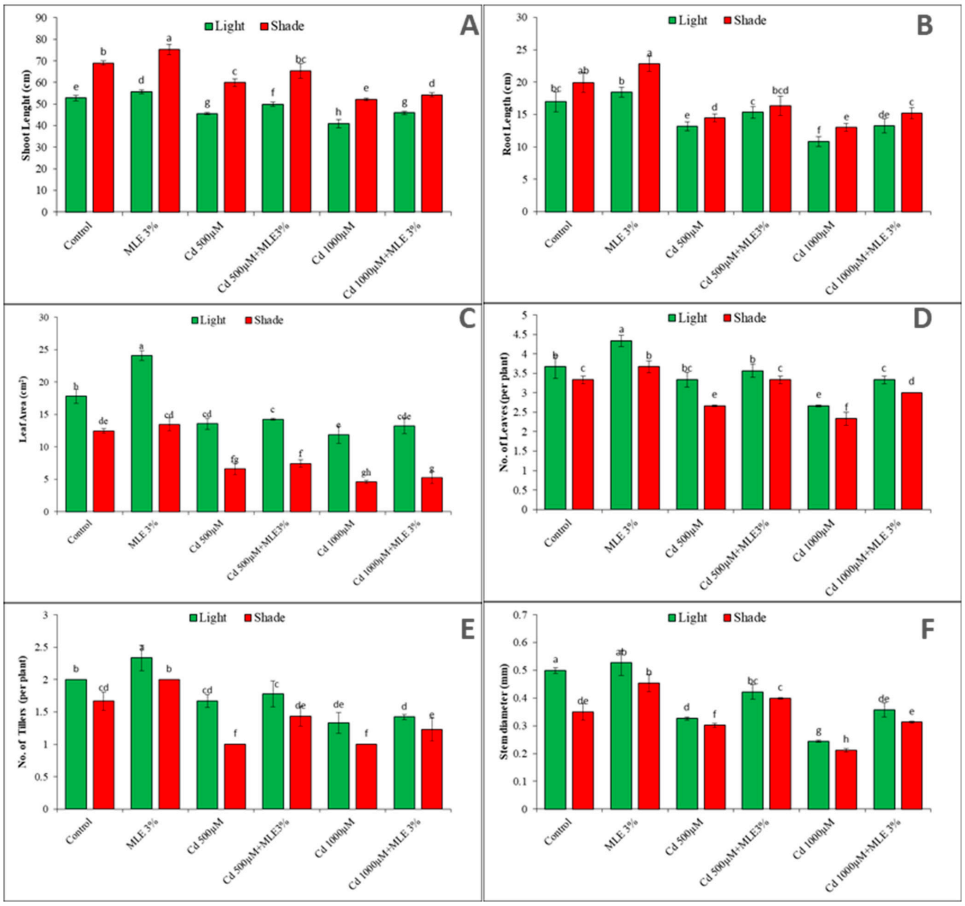


Figure 1. Effect of MLE application on morphological attributes of plant: (**A**) shoot length (**B**) root

length (**C**) leaf area (**D**) Number of leaves (**E**) Number of tillers and (**F**) stem diameter, with and without Cd stress under light and subdue light. Different letters above bars represents significant differences ($p < 0.05$) at each measured point. Values represent mean ± SE of three replicates of each treatment.

3.2. Effect of MLE on Plant Biomass

3.2.1. Shoot Fresh Weight

Figure 2A shows the effect of MLE application on shoot fresh weight of wheat facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) significantly reduced the shoot fresh weight by 81.17% and 118.95% compared to the control, while, on the other hand, application of 3% MLE along with combinations of 500 µM and 1000 µM cadmium solutions decrease the shoot fresh weight by 24.35% and 62.46% compared to control.

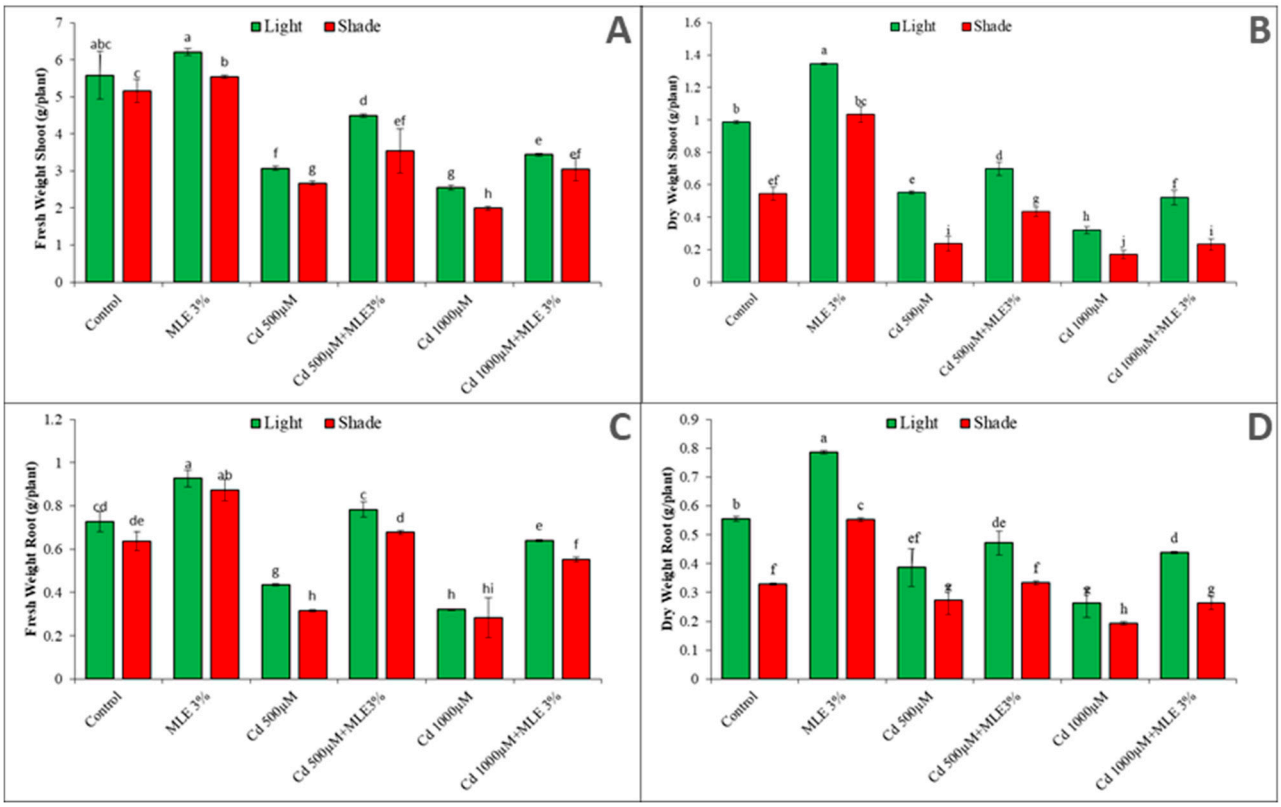


Figure 2. Effect of MLE application on fresh biomass (**A**) shoot fresh weight (**B**) shoot dry weight (**C**) root fresh weight (**D**) root dry weight, with and without Cd stress under light and subdue light. Different letters above bars represents significant differences ($p < 0.05$) at each measured point. Values represent mean ± SE of three replicates of each treatment.

3.2.2. Shoot Dry Weight

Figure 2B shows the effect of MLE application on shoot dry weight of wheat facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) significantly reduced the shoot dry weight by 78.5% and 207% as compared to the control, while on the other hand, application of 3% MLE along with combinations of 500 µM and 1000 µM cadmium solutions increased the shoot dry weight by 7.23% and 0.6%.

More interestingly, 3% MLE with both cadmium treatments even resulted in increased shoot dry weight compared to MLE 3% alone treatment under both light conditions (shade > light).

3.2.3. Root Fresh Weight

Figure 2C shows the effect of MLE application on the root fresh weight of wheat facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) significantly reduced the fresh weight of root by 67% and 83% compared to the control, while, on the other hand, application of 3% MLE along with combinations of 500 µM cadmium solutions increased the root fresh weight by 7.23% while CD 1000 µM+ MLE3% resulted in weight reduction.

3.2.4. Root Dry Weight

Figure 2D shows the effect of MLE application on root dry weight of wheat facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) significantly reduced the dry weight of root by 17.4% and 72.4% as compared to the control, while, on the other hand, application of 3% MLE along with combinations of 500 µM and 1000 µM cadmium solutions increased the root dry weight by 39% and 33% compared to control.

More interestingly, 3% MLE with both cadmium treatments under shade resulted in higher root dry weight compared to MLE 3% alone treatment, while under light reduction in root dry weight was observed.

3.3. Effect of MLE on Plant Pigments

3.3.1. Chlorophyll a Contents

Figure 3A shows the effect of MLE application on chlorophyll a content of wheat facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) significantly reduced the chlorophyll a content by 2.3% and 2%, while, on the other hand, application of 3% MLE along with combinations of 500 µM and 1000 µM cadmium solutions increased the chlorophyll a content by 18.13% and 5.3% compared to respective Cd treatments. While compared to control lower content was observed.

3.3.2. Chlorophyll b Content

Figure 3B shows the effect of MLE application on chlorophyll b content of wheat facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) significantly reduced the chlorophyll b content by 32% and 39%, while, on the other hand, application of 3% MLE along with combinations of 500 µM and 1000 µM cadmium solutions increased the chlorophyll b content of wheat by 10% and 9.9% compared to respective Cd treatments.

3.3.3. Carotenoids

Figure 3E shows the effect of MLE application on carotenoid content of wheat facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) reduced the carotenoid content by 14.7% and 15.7% as compared to control, while application of 3% MLE along with combinations of 500 µM cadmium resulted in 6.7% increase compared to Cd500 µM.

3.3.4. Shoot Anthocyanin

Figure 3C shows the effect of MLE application on anthocyanin content of wheat facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) significantly increased the anthocyanin content of shoot by 56.3% and 60.12% as compared to the control, while, on the other hand, application of 3% MLE along with combinations of 500 µM and 1000 µM cadmium solutions also increased the anthocyanin content by 58% and 61% compared to control.

More interestingly, 3% MLE with both cadmium treatments even resulted in increased shoot anthocyanin compared to MLE 3% alone treatment (higher in shade compared to light).

3.3.5. Root Anthocyanin

Figure 3D shows the effect of MLE application on anthocyanin content of wheat facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) significantly increased the anthocyanin content of root by 61.4% and 68.6% as compared to control, while, on the other hand, application of 3% MLE along with combinations of 500 µM and 1000 µM cadmium solutions also increased the anthocyanin content by 66.38% and 71.4% compared to control.

MLE 3% with both cadmium treatments even resulted in increased root anthocyanin compared to MLE 3% alone treatment (higher in shade compared to light).

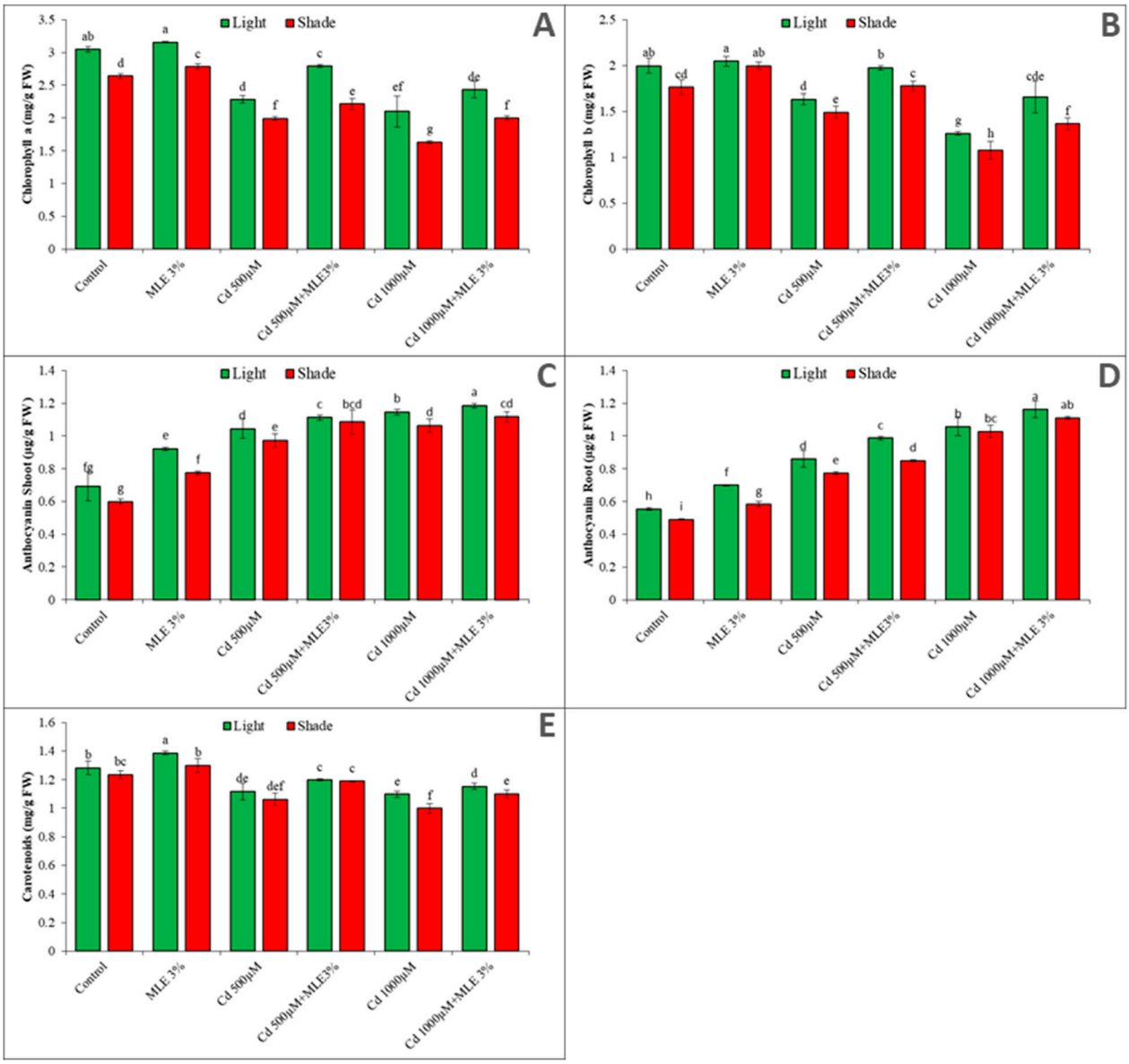


Figure 3. Effect of MLE application on plant pigments (**A**) chlorophyll a content (**B**) chlorophyll b content (**C**) anthocyanin of shoot (**D**) anthocyanin of root and (**E**) carotenoids content, with and without Cd stress under light and subdue light. Different letters above bars represents significant differences ($p < 0.05$) at each measured point. Values represent mean ± SE of three replicates of each treatment.

3.4. Effect of MLE on Reactive Oxygen Species

3.4.1. H_2O_2 Content of Shoot

Figure 4A shows the effect of MLE application on H_2O_2 content of wheat facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) increased the H_2O_2 content by 61.3 and 62.9% as compared to control, while, on the other hand, application of 3% MLE along with combinations of 500 µM and 1000 µM cadmium solutions decreased the H_2O_2 content of wheat by 49.2% and 58.7% compared to control. MLE 3% with both cadmium treatments resulted in increased shoot H_2O_2 Content compared to MLE 3% alone treatment (higher in light compared to shade).

3.4.2. MDA Content of Shoot

Figure 4B shows the effect of MLE application on MDA content of wheat facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) significanlty increased the MDA content by 33.6% and 39% compared to control, while on the other hand, application of 3% MLE along with combinations of 500 µM and 1000 µM cadmium solutions decreased the MDA content of wheat by 6.16% and 23.5% compared to their respive CD treatments.

MLE 3% with both cadmium treatments even resulted in increased shoot MDA Value compared to MLE 3% alone treatment (higher in light compared to shade).

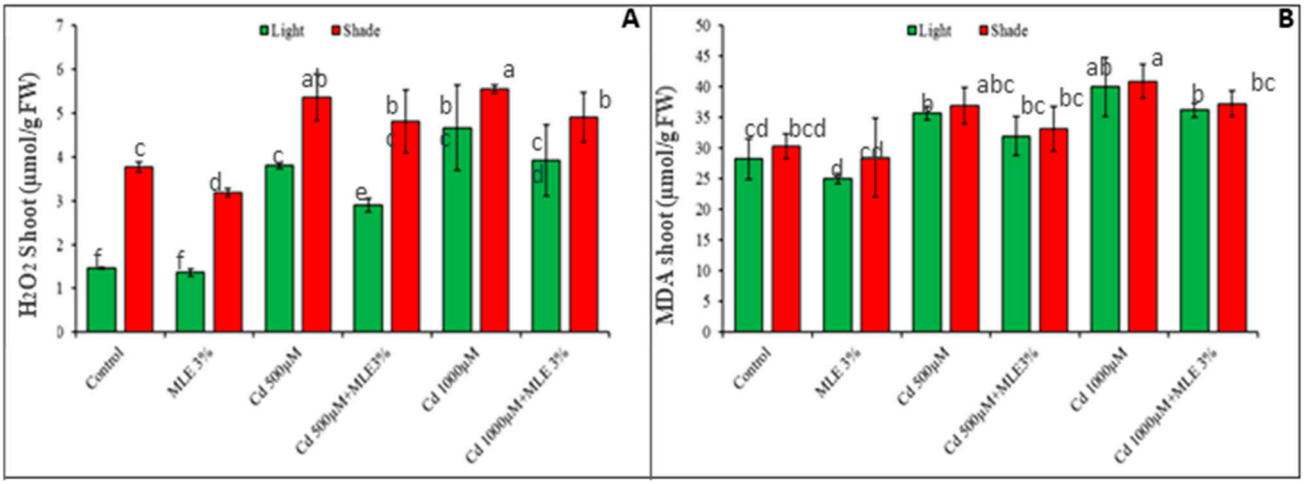


Figure 4. Effect of MLE application on reactive oxygen species (**A**) H_2O_2 measure of shoot (**B**) MDA measure of shoot, with and without Cd stress under light and subdue light. Different letters above bars represents significant differences ($p < 0.05$) at each measured point. Values represent mean ± SE of three replicates of each treatment.

3.5. Effect of MLE on Plant Osmolytes Accumulation

3.5.1. Flavonoids Content of Shoot and Root

Figure 5A shows the effect of MLE application on flavonoids content of wheat facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) significantly increased the flavonoids content of shoot by 32% and 44%, while, on the other hand, application of 3% MLE along with combinations of 500 µM and 1000 µM cadmium solutions also increased the flavonoids content by 33.3% and 44.9% compared to control.

Figure 5B shows the effect of MLE application on flavonoids content of wheat facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) were significantly increased the flavonoids content of root by 11.6% and 26.6%, while, on the other hand, application of 3% MLE along with combinations of 500 µM and 1000 µM cadmium solutions also increased the flavonoids content by 21% and 29% compared to control.

Moreover, both Cd treatments with 3% MLE showed significant increase in root and shoot flavonoids content compared to MLE 3% treatment under both light and shade conditions (light > shade).

3.5.2. Phenolics Content of Shoot and Root

Figure 5C shows the effect of MLE application on phenolics shoot content of wheat facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) were significantly increased ($p < 0.05$) the phenolics content of shoot by 10.9% and 25.56%, while on the other hand, application of 3% MLE along with combinations of 500 µM and 1000 µM cadmium solutions also increased the phenolics content by 22% and 26% compared to control.

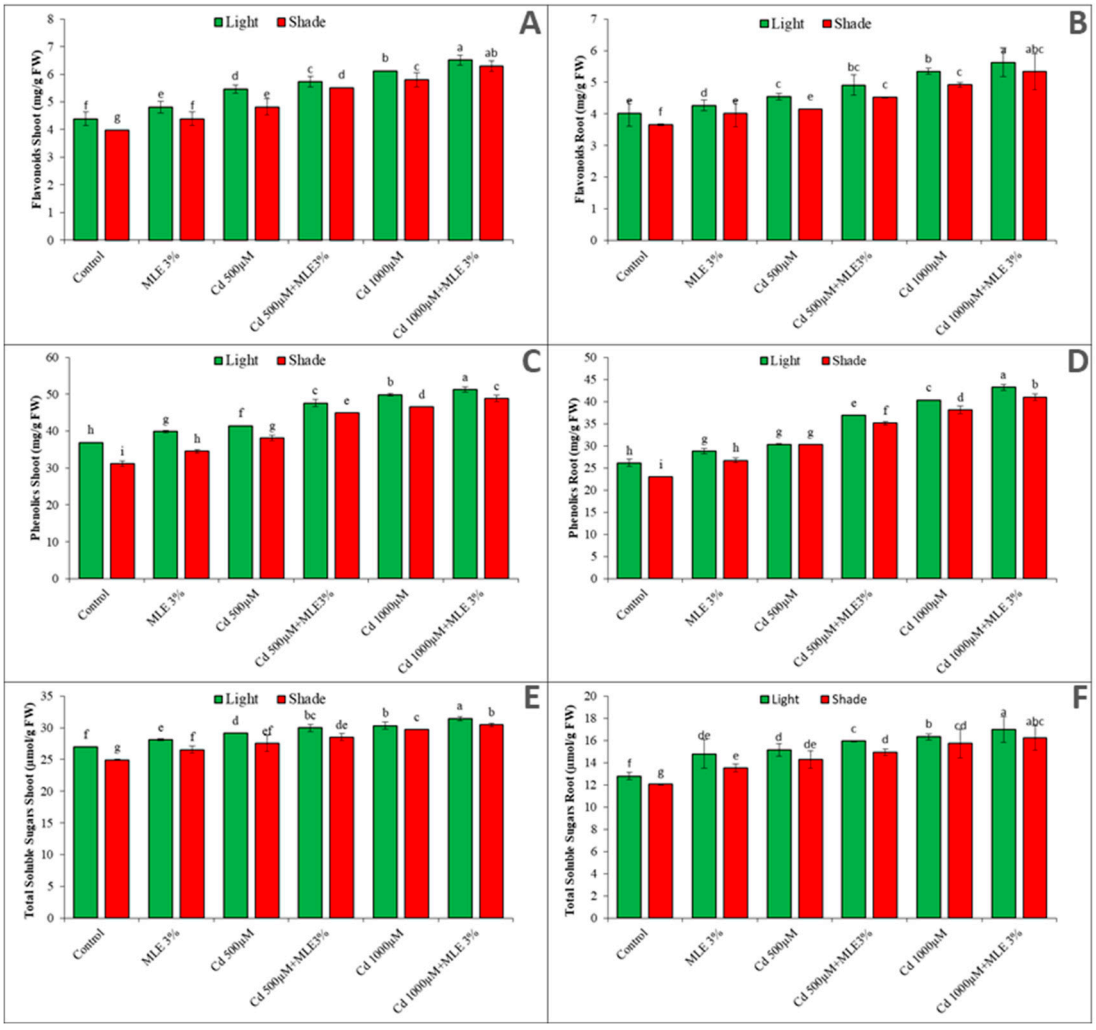


Figure 5. Effect of MLE application on plant osmolytes accumulation (**A**) flavonoids content of shoot (**B**) flavonoids content of root (**C**) phenolics content of shoot (**D**) phenolics content of root (**E**) total soluble sugar content of shoot and (**F**) total soluble sugar content of root, with and without Cd stress under light and subdue light. Different letters above bars represents significant differences ($p < 0.05$) at each measured point. Values represent mean ± SE of three replicates of each treatment.

Figure 5D shows the effect of MLE application on phenolics content of root wheat facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) were significantly increased the phenolics content of root by 13.8% and 36.9%, while, on the other hand, application of 3% MLE along with combinations of 500 µM and 1000 µM cadmium solutions also increased the phenolics content by 29.21% and 47.6% compared to control.

Interestingly, both Cd treatments with 3% MLE showed significant increase in root and shoot phenolics content compared to MLE 3% treatment under both light and shade conditions.

3.5.3. Total Soluble Sugar Content of Shoot and Root

Figure 5E shows the effect of MLE application on soluble sugar content of wheat facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) significantly increased the soluble sugar content of shoot by 7.66% and 6.44%, while, on the other hand, application of 3% MLE along with combinations of 500 µM and 1000 µM cadmium solutions also increased the soluble sugar content by 1.21% and 59% compared to CD stress treatments.

Figure 5F shows the effect of MLE application on soluble sugar content of wheat facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) were significantly increased ($p < 0.05$) the soluble sugar content of root by 20.30% and 19.81%, while, on the other hand, application of 3% MLE along with combinations of 500 µM and 1000 µM cadmium solutions also increased the soluble sugar content by 19.8% and 23% compared to control.

Interestingly, both Cd treatments with 3% MLE showed significant increase in root and shoot total soluble content of sugar, compared to MLE 3% treatment under both light and shade conditions (shade > light).

3.6. Effect of MLE on Mineral Content

3.6.1. Calcium Content of Shoot and Root

Figure 6A shows the effect of MLE application on calcium ion content of wheat shoot facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) significantly reduced the calcium ion content of shoot by 1.8% and 4.9%, while, on the other hand, application of 3% MLE along with combinations of 500 µM and 1000 µM cadmium solutions have not increased the calcium ion content compared to control. Whereas, increase was observed (1.7%) as compared to Cd 500 µM.

Figure 6B shows the effect of MLE application on calcium ion content of wheat roots facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) were significantly reduced the calcium ion content of root by 3.7% and 4.9%, while, on the other hand, application of 3% MLE along with combinations of 500 µM cadmium solutions increased the calcium ion content by 2.16% compared to Cd 500 µM.

Moreover, both Cd treatments with 3% MLE also showed reduction in root and shoot Ca compared to MLE 3% treatment under both light and shade conditions.

3.6.2. Sodium Content of Shoot and Root

Figure 6C shows the effect of MLE application on sodium ion content of wheat shoot facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) significantly increased the sodium ion content of shoot by 8.8% and 27%, while, on the other hand, application of 3% MLE along with combinations of 500 µM and 1000 µM cadmium solutions also increased the sodium ion content by 13.8% and 33.9% compared to control.

Figure 6D shows the effect of MLE application on sodium ion content of root facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) significantly increased the sodium ion content of root by 4.7% and 18.29%, while, on the other hand, application of 3% MLE along with combinations of 500 µM and 1000 µM cadmium solutions also increased the sodium ion content by 9.05% and 24.44% compared to control. More interestingly, 3% MLE without cadmium solution also significantly increased the sodium ion content of root and shoot significantly under both light and shade treatments.

3.6.3. Potassium Content of Shoot and Root

Figure 6E shows the effect of MLE application on potassium ion content of wheat shoots facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM

(under light) significantly reduced the potassium ion content of shoot by 6.4% and 10.47%, while, on the other hand, application of 3% MLE along with combinations of 500 µM solution increased the potassium ion content by 3.58% compared to Cd 500 µM.

Figure 6F shows the effect of MLE application on potassium ion content of wheat roots facing Cd stress. Results revealed that cadmium treatments of 500 µM and 1000 µM (under light) significantly reduced the potassium ion content of root by 5.2% and 9.87%, while, on the other hand, application of 3% MLE along with combinations of 500 µM and solution increased the potassium ion content by 1.54% compared to Cd500 µM. However, with Cd1000 µM +MLE3% there was reduction compared to control in both tissues.

Moreover, both Cd treatments with 3% MLE also showed reduction in root and shoot K compared to MLE 3% treatment under both light and shade conditions.

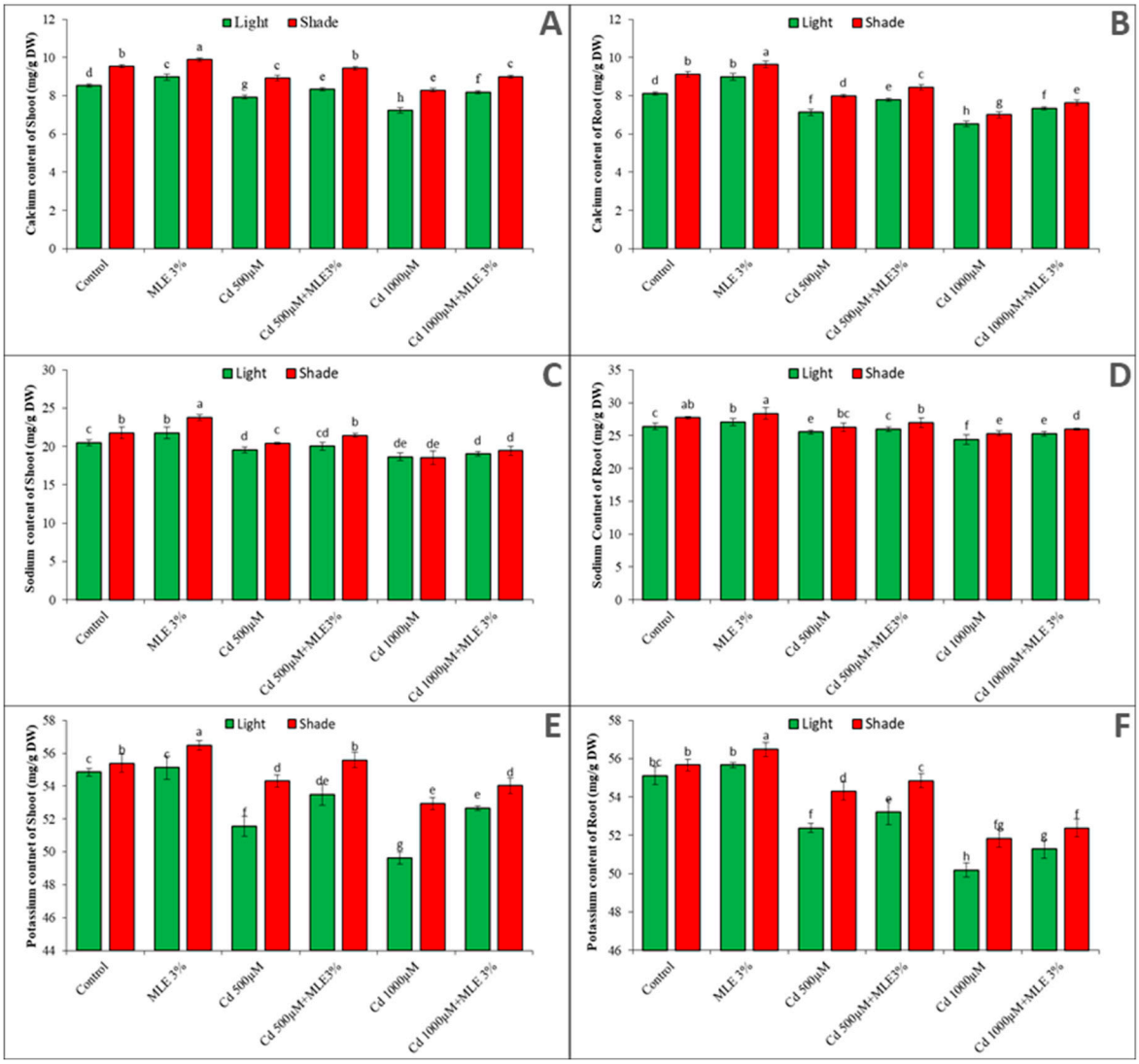


Figure 6. Effect of MLE application on mineral constitutes of plant (**A**) calcium content of shoot (**B**) calcium content of root (**C**) sodium content of shoot (**D**) sodium content of root (**E**) potassium content of shoot and (**F**) potassium content of root, with and without Cd stress under light and subdue light. Different letters above bars represents significant differences ($p < 0.05$) at each measured point. Values represent mean ± SE of three replicates of each treatment.

4. Discussion

Cadmium is one of the most toxic metals that accumulated in different crops through industry and agricultural products. Detrimental effects of Cd also affected the morphophys-

iological and biochemical pathways of wheat, which indirectly influence its growth [23]. Decreasing the uptake of Cd in wheat is one of the potential strategies for minimization of Cd toxicity. In the present study, we demonstrated that soil application of *Moringa oleifera* leaf extract could enhance the growth performance in wheat, also damaging the biological and ecological traits across the growing systems.

Our findings agreed with previous studies. Maishanu et al. [24] revealed that soil application of extract of Moringa significantly enhanced growth of leaves, fresh weight of shoots and dry weight of shoots [24]. Another study revealed that the use of MLE inhibited the biological processes [25]. Another study reported that MLE was found to be a potential biostimulants for enhancing the growth attributes in the wheat. MLE is rich in growth promoting substances like zeatin, indole-3-acetic acid, beta-sitostero, galactinol and cytokine that play an excellent role in cell division, cell elongation, the growth, yield and regulate the biochemical processes in wheat [26].

Cd stress also promotes the degradation of chlorophyll pigments of leaves in wheat [27]. Amirjani et al. [28] revealed that application of moringa leaves extract increased the chlorophyll a and b contents of leaves. They also reported that cytokinin, which is the main component of MLE, promotes the chlorophyll biosynthesis in wheat [28]. Another study reported that treatment of MLE to wheat enhanced chlorophyll contents may also depict its shielding role in downturn of photochemical efficiency with Cd toxicity [29]. Similar observations were depicted in our results that Cd decrease the photosynthetic pigments, and MLE application mitigated this reduction.

Light appears to be an important physical component in regulating a number of biological processes. Shade has an effect on plant morphological, physiological and anatomical features also lowers stem physical strength. Similarly, shading weakens the strength of the stem [30]. Similar results were observed in present research.

Cadmium stress also increased the metabolic pools of the plant metabolism that alleviate the (ROS), hydrogen peroxide (H_2O_2) and malondialdehyde (MDA). The accumulation of ROS species inhibits the antioxidant enzymes that promote the oxidative stress in wheat [31]. Alharby et al. [31] revealed that the application of extract of moringa leaves stimulated the activation of antioxidant enzymes such peroxidases and catalases. They also revealed that these antioxidant enzymes scavenged the ROS and counteracting Cd stress in wheat [31]. Another study reported that treatment of MLE to wheat enhanced the production of secondary metabolites like quercetin-3-O-glucoside, gallic acid, quercetin-3-O-glucoside. The hydroxyl and carboxyl groups of these compounds bind with Cd and inhibit the oxidative stress [32].

Moreover, Basu et al. [33] revealed that the application of extract of moringa leaves increased concentrations of total soluble protein and amino acids, which counteract Cd stress in wheat [33]. Proline, an aliphatic amino acid that reduced the Cd stress in wheat. Dawood et al. [34] revealed that phenylalanine play an important role in activation defense signalling cascades [34]. Similar to observations of current study, a recent study reported effect of MLE foliar spray helped to lower Cd stress by osmolyte accumulation such as flavonoids and proteins essential for the growth of the plant parts [35].

The MLE application proved to be helpful in mitigating the toxic effects of Cd by increasing the growth attributes and mineral content. Further experiments are needed to conduct field experiments in reducing the Cd accumulation in other crop species.

5. Conclusions

In this study, we investigated the antixodant and biological potential of the moringa leaf extract for lowering the uptake and toxicity of Cd, morphological, antioxidant potential and biochemical attributes in a variety A.S2002 *Triticum aestivum* L. Moringa plants are used as biostimulants as they are rich source of antioxidants, amino acids, riboflavin, ascorbic acid folic acid, pyridoxine, vitamins such as A, B and C which are essential for the control of physiological, metabolic, enhanced development, yield attributes, and zeatin that regulate the physiological, biochemical processes and improve the yield under harsh

environmental conditions. Our findings revealed that Cd was involved in the suppression of growth parameters like fresh weight, dry weight, diameter of stem, number of leaves, leaf area etc. Similarly, cadmium stress reduced the photosynthetic pigments, total soluble proteins and total soluble sugars. Cadmium stress increased the hydrogen peroxide (H_2O_2) and malondialdehyde (MDA). The accumulation of ROS species inhibited antioxidant properties in wheat. However, the application of MLE decreased the Cd phtotoxicity and enhanced the osmolytes accumulation under both Cd levels (especially 500 µM level). This approach should be further extended for discovering the molecular mechanisms underlying MLE and host plant interactions. Further experiments are needed to conduct field experiments in reducing the Cd accumulation in other crop species.

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