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Full Length Article

Modulation of Ionic and Water Status by *Moringa oleifera* Extract Against Cadmium Toxicity in Wheat (*Triticum aestivum*)

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Abstract

Wilting and ionic disturbance are consequences of cadmium (Cd) toxicity in plants. The Cd interferes with uptake, transport and utilization of many micro and macronutrients, resultantly caused ionic imbalance. The Cd chloride ($CdCl_2.H_2O$) salt was used to induce Cd stress (0, 500 μM, 1000 μM) at three leaves seedlings stage and at the same time, moringa leaf extract (3%) was foliarly applied on two cultivars of wheat (Faisalabad-08 and Galaxy-13) for maintaining ionic and water status. Data for wheat biomass, water, solute, pressure potential and ionic (calcium, potassium, nitrate, phosphate, sulphate and Cd) attributes were collected at tillering and boot stage of wheat. The inhibitory effect of Cd was more pronounced in root fresh and dry weight. Water and solute potential reduced with Cd stress but elevated significantly with MLE under Cd (500 μM) stress at tillering stage of wheat. Moreover, both wheat cultivars are sensitive to 1000 μM Cd. More accumulation of Ca^{2+}, K^+, NO_3^{2-} and Cd^{2+} ions was observed in root than shoot at both stages. However, the concentration of these ions enhanced in the shoot with MLE at tillering stage. A higher concentration of SO_4^{2-}/S and PO_4^{2-}/P ions was observed in root and shoot samples with Cd (500 μM) and further increase by MLE in shoot samples only. In nut shell, foliar application of MLE served as a biostimulant to ameliorate Cd-induced adverse effects in both wheat cultivars by enhancing mineral nutrients uptake, plants biomass and water relation attributes at both growth stages. © 2018 Friends Science Publishers

Keywords: Boot; Cadmium; Ions; Moringa leaf extract; Tillering; Wheat

Introduction

Wheat (*Triticum aestivum* L.) is a precious crop for the ever-burgeoning world population, and a country must enhance its production to maintain its reliability (FAO, 2009; FAO, 2013). Wheat production in Pakistan was slightly decreased in 2017 (25.1 million tons) than 2016 (25.4 million tons) due to contraction in the planting of rainfed areas followed by long dry season from September to December but still increased as an average result of five years (FAO, 2017). On the other hand, environmental constraints are emerging challenges to the exponentially growing population to maintain and enhance productivity qualitatively and quantitatively (Rizwan *et al*., 2017).

A-biotic stresses like heavy metal contamination through different sources are creating a serious threat to the whole food chain and Cd pollution is an alarming issue in industrial zones (Rizwan *et al*., 2017). Cd being a toxic metal enters into the plants through competing for essential mineral ions by roots and amassed in several plant organs and hampers the crop growth and productivity worldwide (Da-lin *et al*., 2013). Although Cd is not an essential nutrient for plant growth but different crops will take up and accumulate Cd differently depending on its availability in the environment (Grant and Sheppard, 2008). Cd could interfere with the uptake of some mineral nutrients such as iron (Fe) and calcium (Ca) that causes a nutrient imbalance (Astolfi *et al*., 2012; Chang *et al*., 2012). Agricultural wastes are highly toxic, often find a way into the soils and water bodies. These substances cause severe health impacts to humans and aquatic animals (Ivanova *et al*., 2010). Epidemic pieces of evidence suggested that margin of safety between current Cd exposure levels and the threshold for adverse health effects is diminishing sharply (Clemens *et al*., 2013).

The Cd application affects the plant growth by disturbing numerous physiological and biochemical processes like ion toxicity, osmotic stress, nutritional imbalance (Kao *et al*., 2003). Such situation in the plant is partially controlled by showing tolerance mechanism and partially by introducing some osmoprotectants in regulating stress response (Tester and Davenport, 2003).

Various practices are commonly used to alleviate the Cd stress in wheat (Rizwan *et al*., 2017). Osmolytes, organic and inorganic compounds are a potential source for mitigating stress in plants under stress. *Moringa* belongs to family Moringaceae and is rich source of numerous vitamins particularly Vitamin A and C. The *M. oleifera* leaf is a good source of macronutrients [sodium (Na), potassium (K), Ca and magnesium (Mg)] and micronutrients [Fe, Zinc (Zn), Manganese (Mn), Cobalt (Co) and Nickel (Ni)] as well as natural phenolics (Rady and Mohamed, 2015). In various parts of the world, *moringa* tree is called a miracle tree for exhibiting enormous medicinal properties due to the presence of amino acid and phenolics (Yasmeen *et al*., 2013). Deficiency of essential micro and macronutrients was recorded in Cd-treated plants and intake of such food showed toxicity symptoms in human beings. Previous studies supported that MLE mitigated copper (Cu), murcury (Hg), salt and drought stress in different plant species like *Vicia faba* (Kasim *et al*., 2017), *Zea mays* (Bibi *et al*., 2016) and squash plants respectively but effect of MLE yet not been studied on wheat under Cd stress. Thus, this study was conducted to evaluate the effectiveness of MLE in minimizing the Cd-induced adverse effects on nutrients uptake, plant water relations and biomass production of wheat subjected to Cd stress at tillering and booting stage of wheat.

Materials and Methods

A pot experiment was conducted on wheat in the wire house, under natural lighting 29/19±2°C (maximum/minimum mean temperature), of old Botanical garden, University of Agriculture, Faisalabad, Pakistan. Seeds of two wheat cultivars (Faisalabad-08 and Galaxy-13) were collected from Ayub Agricultural Research Institute, Faisalabad. Seeds were surface sterilized with sodium hypochlorite and washed with deionized water thrice before sowing. Leaves of moringa were collected from University of Agriculture, Faisalabad and authenticated from the local experts. Fresh leaves of moringa were washed and stored overnight in -80°C freezer. Frozen leaves were extracted with water in the locally fabricated machine. Moringa extract was filtered through filter paper and diluted up to 3%. A drop of Tween-20 per 100 mL of MLE solution was added as scattering material. Seeds (12) were sown in 22 cm diameter and 32 cm height plastic pots containing 5.5 kg thoroughly washed sand and irrigated with full strength Hoagland's nutrient solution. The seeds were left to grow inside the wire house up to three leaves seedlings of both wheat cultivars. The number of plants per pot were reduced to eight (08) to ensure homogeneity in the experiment. At three leaves seedling stage of both wheat cultivars, Cd (0, 500 μM and 1000 μM) in the form of $CdCl_2.H_2O$ along with MLE was applied under factorial arrangement having completely randomized design (CRD). The data was recorded at tillering and boot stage of both wheat cultivars.

Shoot/root Fresh and Dry Weight (g)

At tillering and boot stage of both wheat cultivars, two shoot and root samples per replicate were pulled out of the sand gently and separately weighed in grams. Fresh weight of shoot and root of each replicate was taken immediately after harvesting and placed them in oven at 72°C until its weight get constant for dry weight of shoot and root.

Water Relation Attributes

Pressure chamber (Scholander-Arimad-2-Japan) was used to record water potential (Ψ_w) in the morning (6-8 a.m.) before the onset of full sunshine at tillering and booting stage of both wheat cultivars. Flag leaf from randomly selected two plants of each replicate was taken for determining water potential. Same leaves were stored in -80°C freezer for 72 h for determination of solute potential (Ψ_s). The cell sap of each frozen leaf was extracted and used to measure osmotic potential with a freezing point osmometer (Capell and Doerffling, 1993). Readings were taken from freezing point osmometer (VAPRO, Model 5520, USA) as nmol/kg and were converted to -MPA. Pressure potential (Ψ_P) was estimated by taking the difference between solute and water potential adopting the protocol devised by Nobel (1991).

$$\Psi_P = \Psi_S - \Psi_W.$$

Digestion of Root and Shoot Samples for Extraction and Analysis

Oven dried shoot and root samples at tillering and boot stage of both wheat cultivars were used for determination of mineral nutrients and Cd concentration. Powdered shoot and root samples (0.1 g) of each replicate were mixed with 5 mL concentrated nitric acid (HNO_3) by adopting the method of Allen *et al*. (1976) with slight modifications. The samples were placed for 3 h at room temperature. After 3 h, shoot and root samples of all replicates were put on the digestion block at 250°C until the solution became tinted yellow in appearance. The digested solution was diluted with 30 mL distilled water and filtered thrice with filter paper.

Estimation of Sulphate (SO_4^{2-}/S) Ions

Sulphate ions were determined by adopting the method of Bai *et al*. (2015) with very slight modifications. The extract was taken from the HNO_3 digested plant samples. To 5 mL of acidic extract of both root and shoot, 0.5 mL of 6N HCl and 0.5 mL of 0.5% (w/v) gum acacia was mixed. The reaction mixture was swirled for 10-15 sec before adding $BaCl_2$ crystals and shacked well until crystals dissolved completely. Absorbance of both shoot and root samples was taken at 340 nm with a UV-spectrophotometer (Model Hitachi-U 2001, Tokyo Japan).

Estimation of Phosphate (PO_4^{2-}/P) Ions

The method of Yoshiba *et al*. (1997) was used to determine phosphate contents in the wheat samples. To 1 mL extract (acid digestion), 2 mL of 2N HNO_3, 2 mL molybdate-vanadate reagent was added and final volume was made to 10 mL with distilled water. Placed the test tubes at room temperature for 20 min. The optical density was taken at 420 nm with the spectrophotometer. Water was used as the blank.

Estimation of Nitrate (NO_3^{2-}/N) Ions

Nitrate ions were determined by following method devised by Sims and Jackson (1971) with slight modifications. To the acidic extract (1.5 mL), 3.3 mL of chromotropic acid was added with a thrust of pipette filler and vortexed briefly. The test tubes were kept at room temperature for 20 min. The intensity of the yellow colour of the reaction mixture was estimated with UV-spectrophotometer at 430 nm. Water was used as the blank.

Estimation of Cations and Cd Ions

Cations (K^+, Ca^{2+}) in root and shoot samples was determined with the flame photometer (Sherwood model 410, UK) from HNO_3 extract. Same acid extract of both root and shoot samples were used to determine Cd^{2+} by atomic absorption spectrum (PerkinElmer, Waltham, MA, USA).

Statistical Analysis

Analysis of variance was analyzed by Statistical Program for Social Sciences (SPSS) software 21 with completely randomized design (CRD) under factorial arrangement having four replicates (Gerber *et al*., 1997). Difference between each treatment at both stages of wheat cultivars was analyzed at $p < 0.05$ by Duncan's Multiple Range Test (DMRT).

Results

Plant Water Relations

The exposure of wheat cultivars with Cd caused significant ($p \leq 0.05$) reduction in water, solute and pressure potential but increased significantly ($p \leq 0.05$) with foliar application of MLE at both stages of wheat cultivars (Table 1). Water potential reduced up to 24-60%, solute potential up to 33-42% and pressure potential greater than 2 fold with Cd stress at both stages. However, foliar application of MLE responds positively and enhanced the water potential by 47-78% at tillering stage and restores the vigor of both wheat cultivars under 500 μM Cd stress in both cultivars. A drastic reduction in water potential (40-60%) was recorded at boot stage. Current data suggested that Cd stress limits the water uptake and imposed osmotic stress in the plant. The foliar application of MLE enhanced the water potential 78% at tillering stage and 2% at boot stage with 500 μM Cd in Galaxy-13 (Fig. 1a). Without Cd stress, exogenous application of MLE enhanced the solute potential by 3-12% (Fig. 1b). Cd treated plants without MLE exhibited reduction to 33-38% at tillering and to 34-42% in solute potential at boot stage in Galaxy-13 and Faisalabad-08 respectively (Fig. 1b). Interestingly, a gradual increase in pressure potential was observed at boot stage (53-65%) under highest level of Cd stress. The Galaxy-13 cultivar of wheat at boot stage showed a more balanced mechanism of water status with Cd stress and foliar application of MLE (Fig. 1c). The wheat cultivar, Galaxy-13 considered more tolerant to Cd stress and responsive to MLE under 500 μM Cd stress (Fig. 1).

Shoot and Root Weight of Wheat

Cd stress significantly ($p \leq 0.05$) reduced seedling vigor in terms of its shoot and root weight (Table 1). Shoot and root fresh/dry weight exhibited significant ($p \leq 0.05$) reduction with both Cd levels but increased by MLE at both stages of wheat cultivars (Table 1). Exogenous application of MLE enhanced shoot fresh weight at both stages than no spray (NS) with or without Cd stress (Fig. 2a). Shoot dry weight reduced up to 2 folds with 1000 μM Cd but the foliar application of MLE showed mitigation of Cd at tillering stage of wheat (Fig. 2b). Both wheat cultivars (Faisalabad-08 and Galaxy-13) showed a non-significant difference for Cd and MLE treatment (Table 1). Root fresh weight reduced to 30-46% with 500 μM and 50-57% with 1000 μM Cd at boot and tillering stage respectively. Exogenous application of MLE enhanced root fresh weight by 35% at tillering and 25% at boot stage under 500 μM Cd stress in both cultivars (Fig. 2c). Root dry weight reduced to 49-54% with 500 μM and 70-85% with 1000 μM Cd at tillering and boot stages respectively (Fig. 2d). The reduction was more prominent for root fresh and dry weight (Fig. 2c and d). It is inferred from the data that 1000 μM Cd is extremely toxic in terms of reducing plant biomass and foliar application of MLE cannot mitigate its adverse effect in both wheat cultivars and stages (Fig. 2b, c and d).

Nutrients and Cd Uptake

The excess of Cd in sand medium disturb the concentration of essential mineral ions in wheat cultivars at both stages (Fig. 3, 4 and 5). Cd and MLE treatments effect significantly ($p \leq 0.05$) shoot and root calcium (Ca^{2+}) and potassium (K^+) concentration (Table 2). Cd negatively correlated to Ca^{2+} and K^+ concentration at both stages of Faisalabad-08 and Galaxy-13 cultivars of wheat (Fig. 3a, b, c and d). It is observed that Ca^{2+} (shoot) reduced up to 44-59% at tillering and 14-22% at boot stage but enhanced with foliar application of MLE (15-25%) at boot stage with increasing level of Cd stress than no spray (NS) group of both cultivars (Fig. 3a).

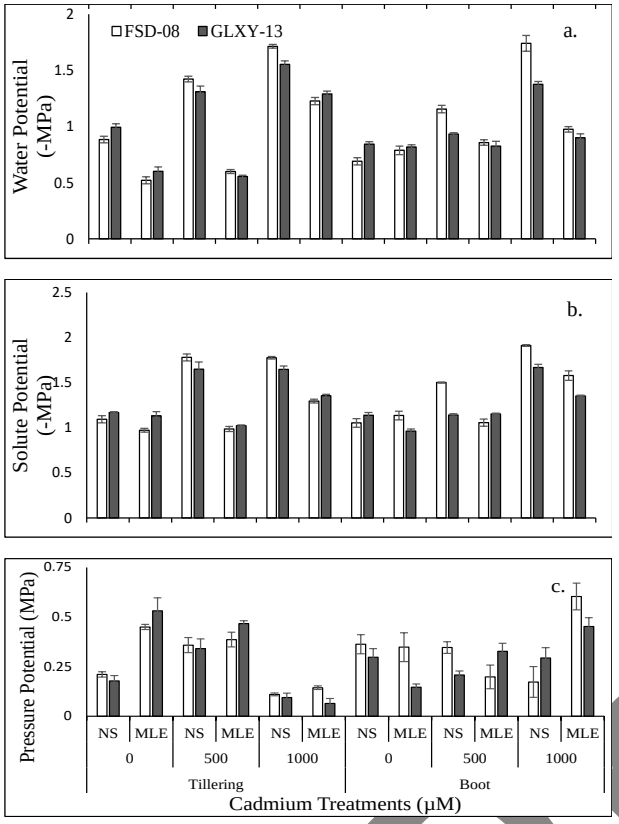

Fig. 1a, b and c: Effect of foliar application of MLE on water, solute and pressure potential of two wheat cultivars under Cd stress ±SE

NS=No Spray, MLE = Moringa leaf extract, FSD-08=Faisalabad-08, GLXY-13=Galaxy-13

Ca^{2+} in root decreased drastically under stress at both stages and application of MLE showed no recovery to increase Ca^{2+} in root of wheat cultivars by mitigating Cd toxicity compare to no NS (Fig. 3b). Potassium (K^+) is essential for normal growth and signaling in plant. According to the current data, there was more accumulation of K^+ at boot stage than tillering stage of both cultivars (Fig. 3c and d). K^+ concentration reduced 20-18% in shoot and 7-37% in root with 1000 μM Cd at tillering and boot stage respectively. However, foliar application of MLE correlate positively with Cd and enhanced K^+ concentration at both stages (Fig. 3c and d). Nitrate (NO_3^{2-}) ion concentration reduced significantly ($p \leq 0.05$) in shoot and root of both cultivars (Table 2). The concentration of NO_3^{2-} in shoot affected drastically by showing reduction up to 13-17% at tillering stage and 43-50% at boot stage with 500 μM Cd and reached to 98% with 1000 μM Cd but its concentration increased 52-69% with foliar spray of MLE at tillering stage of both cultivars than no spray (NS) under 500 μM and 1000 μM Cd stress respectively (Fig. 4a). NO_3^{2-} concentration in root was enhanced with foliar application of MLE (71%) under 1000 μM Cd stress compare to no spray (NS) wheat samples at its tillering stage (Fig. 4b).

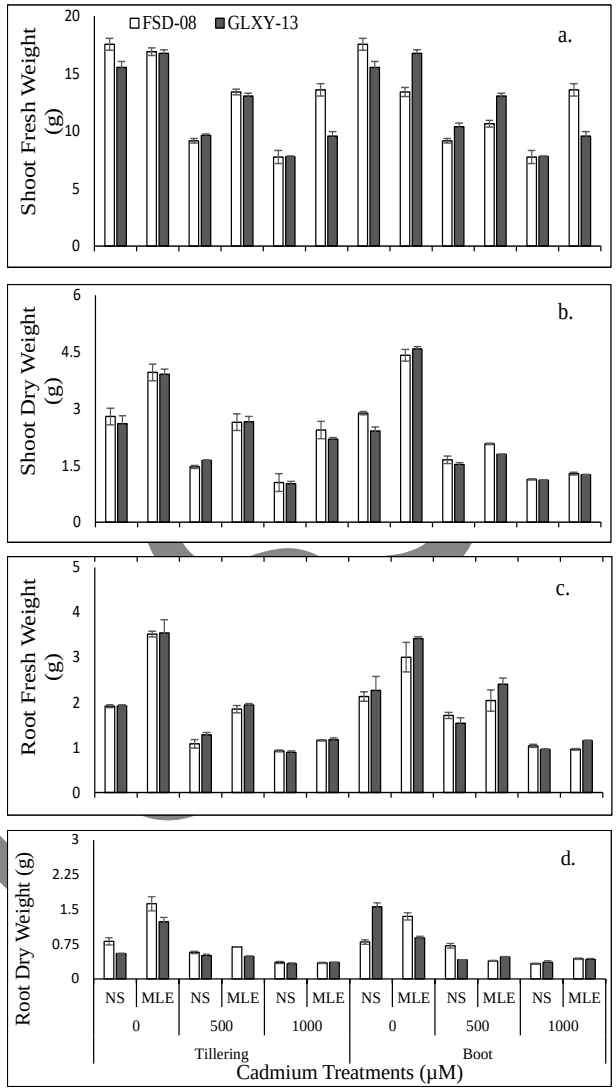

Fig. 2a, b, c and d): Effect of foliar application of MLE on shoot/root fresh and dry weight of two wheat cultivars under Cd stress with ±SE

NS=No Spray, MLE = Moringa leaf extract, FSD-08=Faisalabad-08, GLXY-13=Galaxy-13

Present data suggested that Cd exhibited marked/significant ($p \leq 0.05$) reduction in sulphate ions both in root and shoot samples (Table 3). The concentration of SO_4^{2-} in shoot increased up-to 53% with 500 μM Cd and further threefold increase was observed with foliar spray of MLE at tillering stage of both cultivars while its concentration decreased in root from 20-75% under stress (Fig. 4c and d). A drastic change in SO_4^{2-} of shoot was observed at boot stage of both cultivars, only 3-13% increase in its concentration was observed with exogenous application of MLE in both cultivars at boot stage of wheat. Cd stress was partially mitigated through innate mechanism of wheat (without MLE) by minimizing the reduction rate of SO_4^{2-} concentration (7-21%) under Cd stress.

Table 1: Mean square analysis of variance of root/shoot fresh and dry weight, water, solute and pressure potential of wheat as affected by foliar application of MLE under Cd stress

SOV	DF	Root Fresh weight	Shoot Fresh Weight	Root Dry Weight	Shoot Dry Weight	Water Potential	Solute Potential	Pressure Potential
Cd	2	22.94***	384.45***	0.68***	35.08***	2.79***	1.94***	0.07***
MLE	1	12.07***	101.85***	0.02***	23.70***	3.61***	2.08***	0.21***
Cd*MLE	2	2.68***	43.64***	0.005*	1.81***	0.28***	0.34***	0.03*
Cvs	1	0.22ns	4.323**	2..438e-4ns	0.20ns	0.06***	0.09***	0.01ns
Cd*Cvs	2	0.04ns	17.64***	6.64e-4ns	0.01ns	0.12***	0.06***	0.01ns
MLE*Cvs	1	0.18ns	0.039ns	0.00ns	0.01ns	0.06***	0.07***	7.71e-6ns
Cd*MLE*Cvs	2	0.00ns	29.47***	0.00ns	0.18ns	0.06***	0.07***	0.06***
Stg	1	0.34*	5.04**	0.12***	0.85***	0.10***	0.01ns	0.03*
Cd*Stg	2	0.43**	1.54ns	0.07***	1.22***	0.12***	0.13***	0.36***
MLE*Stg	1	0.56**	8.17***	4.46e-4ns	1.08***	0.38***	0.23***	0.02ns
Cd*MLE*Stg	2	0.14ns	2.04*	0.00ns	1.70***	0.38***	0.13***	0.22***
Cvs*Stg	1	0.05ns	8.17***	4.73e-4ns	0.03ns	0.03**	0.13***	0.02ns
Cd*Cvs*Stg	2	0.05ns	2.04*	5.71e-4ns	0.08ns	0.01*	0.01ns	0.02ns
MLE*Cvs*Stg	1	0.22ns	5.04**	5.71e-4	0.08ns	0.00ns	0.01ns	0.01ns
Cd*MLE*Cvs*Stg	2	0.027ns	1.542**	0.00ns	0.031ns	0.00ns	0.05***	0.03*

Table 2: Mean square analysis of variance of calcium (Ca^{2+}), potassium (K^{+}) and nitrate (NO_3^{2-}/N) ions accumulation in wheat as affected by foliar application of MLE under Cd stress

SOV	DF	Ca^{2+} (Root)	Ca^{2+} (Shoot)	K^{+} (Root)	K^{+} (Shoot)	NO_3^{2-} (Root)	NO_3^{2-} (Shoot)
Cd	2	658.07***	848.13***	863.25***	3769.63***	0.01***	5.05e-4***
MLE	1	3.81*	625.71***	648.54***	2589.95***	0.017***	0.00***
Cd*MLE	2	12.99***	0.19ns	11.67**	6.97**	0.00**	5.83e-4***
Cvs	1	0.04ns	14.44ns	2.136ns	15.80*	9.15 e-6ns	4.94 e-5***
Cd*Cvs	2	2.07ns	30.73*	4.90ns	13.25ns	3.41e-4ns	0.00***
MLE*Cvs	1	0.70ns	0.88ns	10.747*	5.247ns	6.195 e-4ns	7.126 e-4***
Cd*MLE*Cvs	2	0.01ns	3.08ns	2.93ns	12.37*	6.82e-5ns	5.93e-4***
Stg	1	0.63ns	1760.19***	50.16***	4742.23***	0.01***	4.37e-4**
Cd*Stg	2	43.32***	56.39**	47.30***	227.72***	3.12e-4ns	0.01***
MLE*Stg	1	1.50ns	64.86*	30.31***	6.71ns	0.01***	3.94 e-4**
Cd*MLE*Stg	2	0.723ns	0.64ns	28.29***	139.85***	2.06e-4ns	0.00***
Cvs*Stg	1	3.96e-4	33.09ns	14.871*	19.161ns	6.214e-4ns	0.002***
Cd*Cvs*Stg	2	2.94*	4.65ns	6.50ns	60.70*	0.00**	9.26e-4***
MLE*Cvs*Stg	1	2.39ns	5.80ns	3.20ns	83.30*	2.71e-4ns	8.61e-4***
Cd*MLE*Cvs*Stg	2	4.75*	0.72ns	3.17ns	3.48ns	2.56e-4	3.77e-4***

Table 3: Mean square analysis of variance of sulphate (SO_4^{2-}/S), phosphate (PO_4^{2-}/P) and cadmium (Cd^{2+}) ions accumulation in wheat as affected by foliar application of MLE under Cd stress

SOV	DF	SO_4^{2-} (Root)	SO_4^{2-} (Shoot)	PO_4^{2-} (Root)	PO_4^{2-} (Shoot)	Cd^{2+} (Root)	Cd^{2+} (Shoot)
Cd	2	0.02***	0.00***	0.023***	0.005***	43881.48***	12702.8***
MLE	1	3.93e-5ns	0.01***	3.384e-7ns	9.64e-4***	656.56***	537.94***
Cd*MLE	2	3.53e-5ns	3.45e-4***	1.103e-4*	0.001***	161.28***	131.81***
Cvs	1	1.034e-4ns	3.62e-6ns	3.488e-6ns	6.165e-5**	9.69*	66.25*
Cd*Cvs	2	5.36e-5ns	1.31e-4**	9.315e-5ns	5.185e-6ns	19.56***	26.79ns
MLE*Cvs	1	1.14e-5ns	1.68e-4*	5.088e-6ns	3.31e-5*	38.76***	8.91ns
Cd*MLE*Cvs	2	6.05e-5ns	1.13e-4*	5.268e-5ns	2.95e-6ns	81.22***	30.63ns
Stg	1	0.01***	0.00***	9.747e-4***	8.59e-4***	3644.50***	10.50ns
Cd*Stg	2	0.01***	0.00***	1.319e-4*	1.775e-5ns	1130.65***	52.21*
MLE*Stg	1	1.32e-6ns	2.03e-4**	5.813e-5ns	4.60e-4***	198.38***	239.88***
Cd*MLE*Stg	2	1.02e-4*	2.17e-4***	7.221e-5ns	1.34e-4***	53.02***	77.52**
Cvs*Stg	1	3.98e-5ns	6.23e-5ns	6.355e-6ns	3.39e-5*	0.44ns	71.329*
Cd*Cvs*Stg	2	1.76e-4**	3.21e-5ns	4.129e-5ns	1.99e-5ns	1.24ns	52.52*
MLE*Cvs*Stg	1	1.29e-5ns	8.66e-6ns	4.550e-6ns	9.66e-7ns	0.67ns	2.58ns
Cd*MLE*Cvs*Stg	2	7.77e-6ns	1.07e-4*	8.817e-5ns	1.55e-5ns	6.51ns	8.52ns

Note: Cd= Cadmium, MLE= Moringa leaf Extract, Cvs= Cultivars, Stg= Stages

Interestingly, the concentration of SO_4^{2-} ions in root was higher with 1000 μM Cd with and without foliar application of MLE at boot stage of both cultivars. Phosphate ion (PO_4^{2-}/P) concentration enhanced significantly ($p \leq 0.05$) with Cd stress in root and shoot of wheat cultivars, while MLE exogenous application caused slightly decreased in PO_4^{2-} concentration. A non-significant difference was recorded for foliar application of MLE in root of both wheat cultivars. (Table 3). Data showed that PO_4^{2-} concentration in shoot enhanced linearly with increasing Cd stress (Fig. 5a) and suppress with foliar application of MLE in shoot samples at both stage of wheat cultivars (Fig. 5a). PO_4^{2-} in root accumulated more than 90% at tillering stage and 85% at boot stage under both levels of stresses (Fig. 5b).

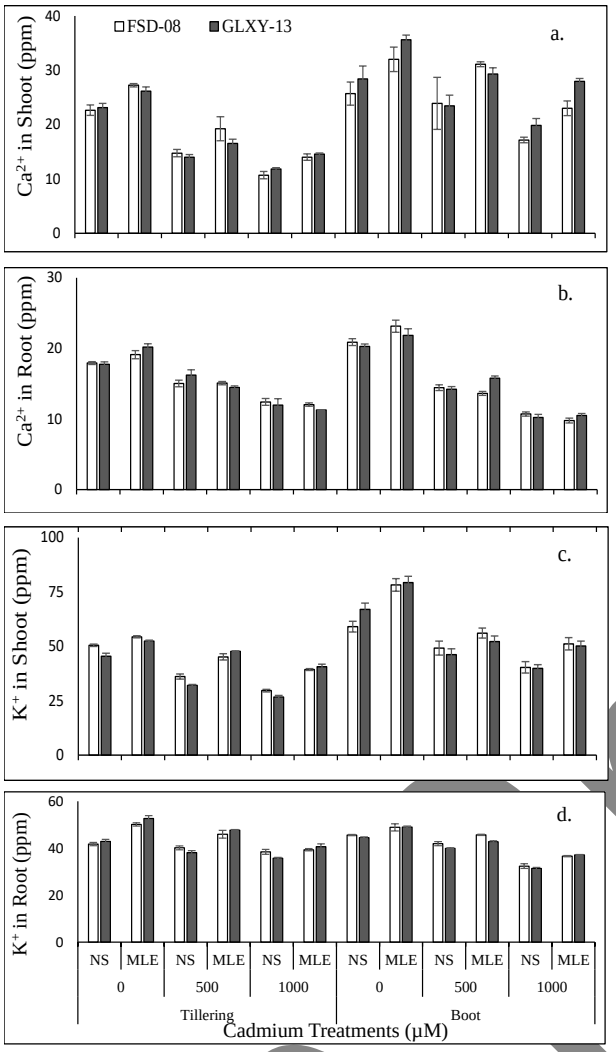

Fig. 3a, b, c and d: Effect of foliar application of MLE on calcium (Ca^{2+}) and potassium (K^+) ions of two wheat cultivars under Cd stress with ±SE

NS=No Spray, MLE = Moringa leaf extract, FSD-08=Faisalabad-08, GLXY-13=Galaxy-13

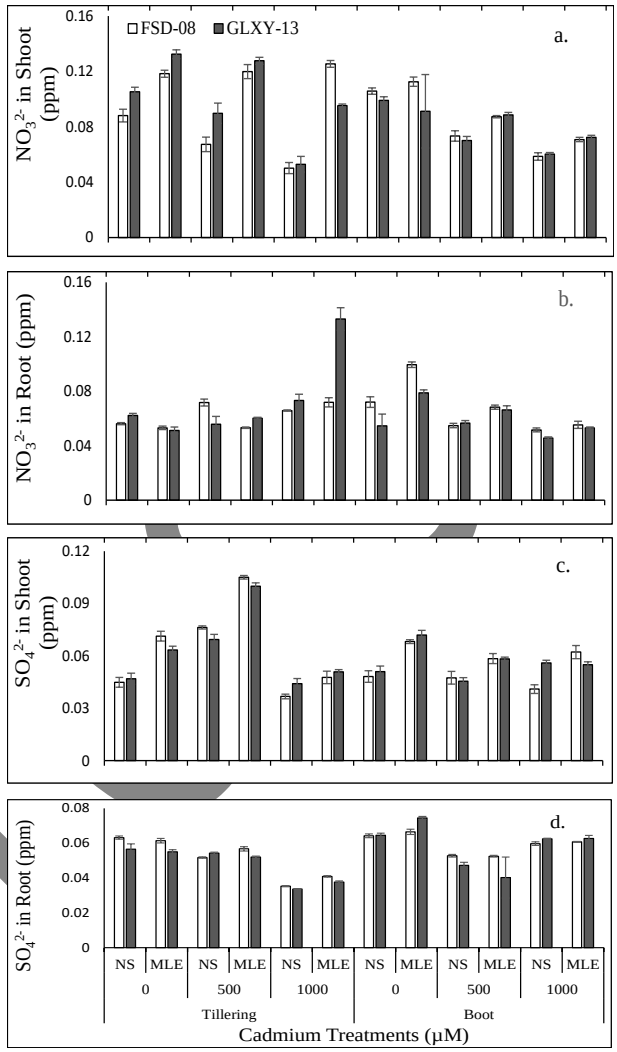

Fig. 4a, b, c and d: Effect of foliar application of MLE on nitrate (NO_3^{2-}) and sulphate (SO_4^{2-}) ions of two wheat cultivars under Cd stress with ±SE

NS=No Spray, MLE = Moringa leaf extract, FSD-08=Faisalabad-08, GLXY-13=Galaxy-13

A differential expression was observed for the accumulation of Cd in root and shoot of both wheat cultivars (Fig. 5c and d). A significant ($p \leq 0.05$) and linear increase of Cd^{2+} concentration in the shoot was observed with increasing level of stress at both stages of wheat cultivars. However, foliar application of MLE caused a significant reduction in Cd^{2+} concentration both in root and shoot in both cultivars and stages (Table 3). Foliar application of MLE reduced Cd^{2+} concentration in shoot 52-64% under 500 μM Cd and 10-25% under 1000 μM Cd at boot and tillering stage respectively of both cultivars than no spray (NS) which showed 98-200% reduction (Fig. 5c). A significant increase in Cd^{2+} concentration was observed with increasing Cd stress in roots of both cultivars particularly at boot stage (Table 3). It is evident from current data that prolonged exposure to Cd produced more deleterious effects on roots of both wheat cultivars but mitigation effect of MLE was recorded under levels of stress (Fig. 5d). It was also recorded that root showed more accumulation of Cd^{2+} than shoot and may have locked many essential nutrients in the root as well (Fig. 5c and d).

Discussion

Cd caused an extreme reduction in water and osmotic potential. However, under normal condition, passive movement of water into root by transpiration pull maintains the upward movement of water through xylem vessels (Carvajal *et al*., 1996; Renata, 2016).

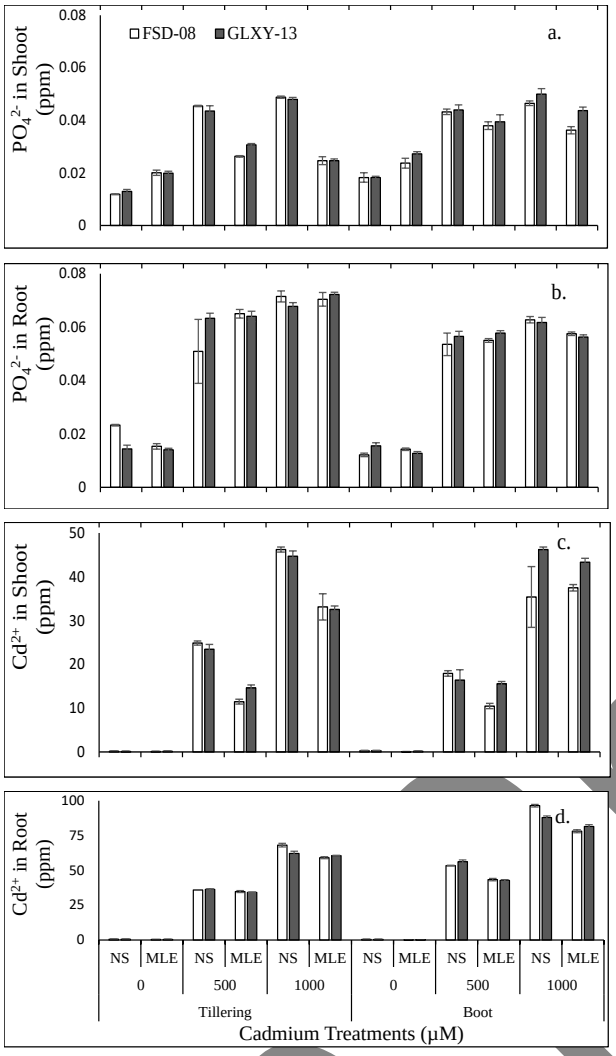

Fig. 5a, b, c and d: Effect of foliar application of MLE on phosphate (PO_4^{2-}) and Cd of two wheat cultivars under Cd stress with ±SE

NS=No Spray, MLE = Moringa leaf extract, FSD-08=Faisalabad-08, GLXY-13=Galaxy-13

But an excess of Cd had hindered the passive flow of water, thus influenced the water potential which indirectly affected solute and pressure potential and same phenomenon has been reported in the present work conducted on two wheat cultivars (Fig. 1). It was also reported that long-time exposure to Cd caused decreased water contents by decreasing essential mineral contents. Moringa oleifera leaf extract is rich source of osmoprotectants (*i.e.*, soluble sugars, free proline and free amino acids), mineral nutrients like macro (N, P, K, Ca, Mg and) and micronutrients (Fe, Cu, Zn and Mn), antioxidants and vitamins (*i.e.*, salicylic acid, α-tocopherol, that may serve as restoration of water, solute and pressure potential in both wheat cultivars (Ali *et al.*, 2017). It is likely that water entry to the roots is indirectly governed by other factors which are themselves affected by metals. It was suggested by Ali *et al*. (2017) that some of these mineral nutrients present in MLE, if not all can easily translocate to active parts of plants and thus mitigated stress and enhanced the water relation attributes in both wheat cultivars under Cd stress.

The reduction of water related attributes had also negatively affected shoots and root fresh and dry weight under excess of Cd in the current study. This loss of turgidity also disturbs the primary function of root by modulating concentration of essential mineral nutrients and at the same time, it is the first site of contact with Cd which collectively posed a heavily negative impact on root growth and same effects were recorded by other scientists (Renata, 2016). Chaudhary and Panda, (2004) also reported that Cd served as an inhibitor for cell division and cell elongation which resultantly reduced the plant biomass. The reduction in wheat biomass may be attributed by direct and indirect impact of Cd on photosynthesis, gas exchange parameters as well as nutritional imbalance (Shah *et al.*, 2011). However, the negative effect of Cd was alleviated with MLE under Cd (500 *µ*M) stress and enhanced root and shoot fresh weight (Fig. 2a and b). The significance of MLE proved to be effective in improving root and shoot fresh weight of *Capsicum annuum* L. (Hala *et al.*, 2017). The improvement of shoot and root biomass might be due to efficacy of MLE to improve photosynthetic ability and scavenging reactive oxygen species under saline stress or may be by adjusting its water and solute potential (Zhang and Ervin, 20004) as previously reported by Yasmeen *et al*. (2013) that exogenous application of MLE enhances the leaf area and photosynthetic rate in wheat under salt stress. Mitigation of Cd by MLE may be attributed to the presence of strong growth promoting substances like Zeatin, Purine, adenine and cytokinin in its extract as suggested by Muhammad *et al.* (2015). Growth inhibition in the current study was associated with increasing concentration of Cd in plants vicinity. This result correlated positively with the study of Mostafa and Hamida (2015).

A significant decrease in various ions was observed with increasing Cd stress in the present study. This effect is also consequence of reduction in water uptake and increase in Cd translocation instead of essential mineral contents by using Zn-Fe transporters. Cd influence plants physiological and biochemical processes by interfering essential mineral nutrients that in another case may provide shielding effect against Cd toxicity (Khan *et al*., 2007). The previous study of Semida *et al*. (2015) also suggests that Cd limits some physiological constraints and decrease the plant vigor. Tkalec *et al.* (2014) exhibited that Cd significantly reduced Ca^{2+} in pea plant. MLE possesses a good amount of mineral nutrients as reported by Yameogo *et al.* (2011) which may have boosted the accumulation of K^+ and Ca^{2+} concentration under Cd stress in both root and shoot of tillering and boot stage of wheat. Yasmeen *et al.* (2013) also reported positive effect in wheat with MLE and increased K^+ conc. under drought stress and the same mechanism may prove significant with Cd stress.

This nutritional imbalance may cause a reduced number of leaves, stunted growth and cell elongation (Sivritepe *et al*., 2003). It is concluded that Cd at the rate of 1000 μM significantly reduced Ca^{2+} and K^{+} in wheat as previously reported by Quzounidou *et al.* (1997). A sharp reduction in these ions (Ca^{2+}, K^{+}) exhibited their efflux across the cell membrane under excess dosage of Cd, thereby enhancing MDA contents as a result of toxicity in the cytosol (Janas *et al*., 2010). Current data suggested that NO_3^{2-} reduction by Cd stress, same result was reported by Mujtaba *et al.* (2013). Nitrate ions concentration was enhanced with the foliar spray of MLE under an excess of Cd. MLE is the rich source of amino acid that can be positively correlated to the enhancement of NO_3^{2-} concentration under stress (Fuglie, 2001). NO_3^{2-} concentration both in shoot and root may help in activating various enzymes involved in carbohydrate and nucleic acid metabolism which played a significant role in mitigating Cd toxicity (Bednarz and Oosterhuis, 1999). An increase in Cd stimulated the accumulation of SO_4^{2-} in the root that positively correlated for stimulation of sulfur in the shoot of both wheat cultivars with 500 μM Cd stress and the same result was reported previously (Nocito *et al*., 2006). Sulfur is an important nutrient and end product of sulphur assimilation is cysteine which serves as a precursor for phytochelatin biosynthesis for detoxification of Cd (Wu *et al*., 2006). The concentration of SO_4^{2-}/S in the shoot is particularly enhanced by foliar spray of MLE. Previous reports suggested that aqueous extract contains rich amount of sulphur (870 mg/100 g of dry leaves), might be involved in mitigation Cd stress in both wheat cultivar (Thanaa *et al*., 2017). An increase in phosphate contents with Cd stress was observed in the current study that PO_4^{2-}/P contents enhanced with Cd. It was suggested by Arshad *et al*. (2016) that application of phosphorus decrease uptake of Cd. Our result negatively correlates with Fuglie (2001) who showed that MLE possess a good amount of phosphorus but the foliar application of MLE caused a reduction in PO_4^{2-}/P contents in the current study. The exogenous application of salicylic acid enhanced N, P concentration in wheat which may be proved true for exogenous application of MLE (Gunes *et al*., 2005).

A differential mode of Cd accumulation was observed in both organs (root and shoot) of wheat cultivars and prolonged exposure also displayed more damaging effects in wheat as evident in current research. Previous reports suggested that Cd uptake is enhanced with increasing level of Cd stress (Dunbar *et al*., 2003; Wang *et al*., 2009). It was also suggested that Cd contents unevenly distributed in various organs and tissues of plants. There was more accumulation of Cd in root than shoot as suggested in previous studies (Durand *et al*., 2010). Foliar application of MLE caused the reduction on tissue Cd both in shoot and root. A study of Papoh *et al*. (2011) also demonstrated that MLE has the capacity to reduce Cd in Cd polluted water. According to Yasmeen *et al*. (2013), MLE decreased shoot Na^{+} and Cl^{-} with simultaneous increase of K^{+} concentration in both wheat cultivars at tillering and boot stage. As MLE is effective against salt stress, so Cd being abiotic stress can also be mitigated using the same mechanism.

Conclusion

Cd adversely affected wheat growth in terms of reduced root and shoots biomass along with a reduction of essential mineral nutrients and increase in Cd accumulation at both stages of wheat cultivars. Moringa is a natural and eco-friendly and economical source for enhancing Cd tolerance in wheat as observed in current experiment. MLE significantly enhanced mineral nutrients uptake along with homeostatic equilibrium of water, solute and pressure potential under an excess of Cd in wheat.

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