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MORINGA LEAF EXTRACT AND ASCORBIC ACID EVOKE POTENTIALLY BENEFICIAL ANTIOXIDANTS ESPECIALLY PHENOLICS IN WHEAT GROWN UNDER CADMIUM STRESS

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Abstract

Moringa oleifera (Sohanjna) is considered as a miraculous plant for pertaining a handsome blend of growth promoting substances. The glistening effects of moringa leaf extract (MLE) and ascorbic acid (AsA) were investigated at two stages of wheat grown under cadmium (Cd) stress. The cadmium chloride ($CdCl_2.5H_2O$) was used to induce Cd stress (0, 500 µmol/L, 1000 µmol/L) at three leaf seedlings stage along with foliar spray of moringa leaves extract (3%) and ascorbic acid (50 mmol/L). Data was collected at two stages (Tillering and Boot) of wheat. The activities of superoxide dismutase (SOD), guaiacol peroxidase (GPX), catalase (CAT), ascorbate peroxidase (APX) and total soluble protein (TSP) increased significantly with foliar spray of MLE and AsA at boot stage of wheat under Cd (500 µmol/L) stress, while elevated level of Cd irreversibly damage antioxidant response by accumulating higher amount of hydrogen peroxide (H_2O_2), malondialdehyde (MDA) contents. Furthermore, quercetin, gallic acid, caffeic acid, vanillic acid, chlorogenic acid and coumeric acid confers tolerance with MLE and AsA under stressful conditions at both stages while syringic and sinapic acid were the least expressed phenolic compounds in wheat. MLE is an effective remedy to mitigate oxidative damage caused by Cd and enhanced enzymatic and non-enzymatic antioxidant response in wheat particularly at boot stage.

Key words: Abiotic stress; Aqueous; Ascorbate; Natural stimulant; Secondary metabolite.

Introduction

The metal load in plants is an abiotic stress which is expanding with exponentially growing population and industries, hence deteriorating agricultural soil with poor cereals production in terms of quality and quantity (Asopa *et al.,* 2017). During respiration carbonic acid in the soil (H_2CO_3) breaks down into proton (H^+) and bicarbonate ions (HCO_3^-). These absorbed protons (H^+) are quickly exchanged with Cd^{2+}, hence ensures its entry from rhizosphere into the epidermal root cells through apoplast pathway (Yamaguchi *et al*., 2011). Cd via symplastic pathway carried by Zn-Fe and Ca^{2+} channels in a concentration dependent manner (Sadana *et al*., 2003). Cadmium is highly toxic and limit growth of plants (Iftikhar *et al*., 2012). A high dose of Cd disturbs the ionic status of plant cell (Fozia *et al.,* 2018) including ferric ions (Fe^{3+}), which serve as cofactor for catalase (CAT) and peroxidases (POD) to scavenge oxidant species (Tchounwou *et al.,* 2012). Under stress, roots actively secrete low molecular weight compounds to enhance absorption of Cd^{2+} from the rhizosphere into the plant's cell (Curie *et al*., 2009). Under control and mild stress conditions, a cellular antioxidant defense system like catalase, peroxidase and superoxide dismutase is mounted with time in plant to reduce effect of reactive oxygen species (Jozefczak *et al.,* 2015). Secondary metabolites are intensively synthesized compounds under abiotic stress (Nascimento & Fett-Neto, 2010; Arneeb & Shahbaz, 2020) which elevate the immunity of the plant against ROS as well as lipid peroxidation (Allakhverdiev *et al*., 2008). Thus, non-biological stress may cause reduction or enhance the contents of phenolic compounds in the plants's cell. Phenolics may form complexes with heavy metals to scavenge ROS. Phenolics compounds could be sum up as the determinant of antioxidant potential in wheat to survive under stress (Weidner *et al.,* 2009). Uncontrolled oxidation induced by reactive oxygen species (ROS) can be alleviated by effective strategies induced by the innate mechanism of plants (Hippler *et al.,* 2015). Extracts of different plants with high phenolic content have been in great demand of food industry because they delay oxidative decomposition of lipids and thus enhance the antioxidant potential of economically important crops (Jorge *et al*., 2019)**.** *Moringa oleifera* is a well-known local tree of Pakistan and proven as the potential source of chemicals to mitigate different abiotic stresses (Yasmeen *et al.,* 2012). Fresh moringa leaves (100g) contains a good combination of protein (6.7 g), fats (1.7 g), carbohydrate (12.5 g), fiber (0.9 g), vitamin C (220 mg), Vitamin E (440 mg), magnesium (42 mg), phosphorus (70 mg), potassium (259 mg), copper (1.1 mg) and iron (0.85 mg) (Dhakar and Maurya 2011). Various phytochemicals and anticancerous compounds such as sterols, terpenes, flavonoids, saponins, anthraquinones, alkaloids, sugars, glucosinolates, isothiocynates, glycoside and glycerol-1-9-octadecanoate are also present in moringa leaf extract (Berkovich *et al.,* 2013). With the soul significance of moringa, current research was aimed to

assess role of *Moringa oleifera* aqueous extract in comparison to ascorbic acid as a reference molecule under cadmium stress at different physiological stages (Tillering and Boot) of wheat. *Moringa oleifera* should be discussed in more depth to evaluate impact of particular component/ components to mitigate abiotic stress in wheat. Here is a useful contribution for describing antioxidants as a marker against Cd stress.

Materials and Methods

The experiment was laid down in completely randomized design in the net-house of old Botanical Garden, University of Agriculture, Faisalabad, Pakistan. The wheat cultivar (Faisalabad-08) was kindly provided by Ayub Agriculture Research Institute (AARI). Prior to seed sowing, seeds were treated with 70% ethanol (5-7 min.) and sodium hypochlorite (2-3 min), then rinsed thoroughly with deionized water thrice and let them dry completely. Seeds (07) were sown in malleable pots having thoroughly washed dry sand. Seeds were supplemented with full strength Hoagland nutrient medium and allowed to grow upto three leaf seedling stage before the application of treatments.

Preparation and applications of treatments: Leaves of moringa were plucked from tress of *Moringa oleifera* near department of Forestry, University of Agriculture, Faisalabad and authenticated by local experts. All the dust and other contaminants on leaves was removed by gentle washing with tap water repeatedly and let it dry for 2-3 h(hours) in shade. Leaves were weighed with electric balance and kept at -80^{o}C for 72 h. Concentrated extract was made with a locally fabricated machine and diluted with deionized water to 3%. Ascorbic acid was purchased from Merck-Aldrich. A standard stock solution of 200 mmol/L was made and diluted to 50 mmol/L for foliar application to wheat seedlings by adding tween-20. Cadmium Chloride ($CdCl_2.5H_2O$) was also purchased from Merck-Aldrich. The stock solution of Cd was made and diluted as 0, 500, 1000 µmol/L concentrations and applied in the rhizosphere of wheat seedlings. All treatments were repeated once a week upto four weeks having four replicates. The completely randomized design of the experiment was as follows:

Group 1	Group 2	Group 3
Control (0 µmol/L Cd)	500 µmol/L Cd	1000 µmol/L Cd
Control + AsA	500 µmol/L Cd + AsA	1000 µmol/L Cd + AsA
Control + MLE	500 µmol/L Cd + MLE	1000 µmol/L Cd + MLE

The following attributes were studied at tillering and boot stage of wheat after 30 and 60 days of treatments respectively

Determination of malondialdehyde (MDA) and H_2O_2 contents: Heath and Packer (1968) method was adopted to analyze MDA contents in known weight of fresh leaf sample. Fresh leaves were ground in 1% w/v trichloroacetic acid (TCA) and swirled at 10,000 g for 10 min and the supernatant was transferred into another Eppendorf. A homogenous solution of 0.5% thiobarbituric acid (TBA) was freshly prepared in 20% TCA, mixed with TCA supernatant. The resultant solution was boiled (95°C) for 30 min in water bath. The optical density (OD) of the solution was recorded at 532 nm and 600 nm. The optical density taken at 600 nm was used to correct by subtracting the non-specific absorbance. Previously ground and centrifuged solution (1% TCA) was diluted (0.1%) to measure H_2O_2 activity in wheat seedlings (Velikova *et al.,* 2000). The absorbance was taken at 390 nm with spectrophotometer (IRMECO U2020).

Extraction and assessment of antioxidant enzymes: Enzyme extraction was made with fresh leaves of wheat (0.25 g) in ice-cold potassium phosphate buffer (pH 7.8) (Mukherjee & Choudhuri, 1983). After grinding, crude liquid sample was centrifuged at 15000 g (20 min) at 4°C to separate enzyme layer. The enzyme extract was stored in 500 µl aliquots at 4°C for antioxidant analysis. Catalase (EC 1.11.1.6) activity was estimated from the enzyme extract, with the addition of 50 mmol/L buffer (pH 7.0) and 35% v/v H_2O_2 (Aebi, 1984). The decrease in optical density (OD) was recorded at 240 nm for 2min with an interval of 20 seconds. Guaiacol peroxidase (GPX; EC 1.11.1.7) activity was determined by using guaiacol reaction mixture containing 40 mmol/L H_2O_2, 20 mmol/L guaiacol and 0.5 mL enzyme extract (Maehly & Chance, 1954). Change in abosrbance was measured with spectrophotometer at 470 nm. Ascorbate peroxidase (APX) activity (EC 1.11.1.11) was evaluated by using method of Chen & Asada (1989). The reaction mixture contained (3 mL) 0.5 mmol/L ascorbic acid, 0.5 mmol/L H_2O_2, 50 mmol/L phosphate buffer and 0.5 mL enzyme extract. The optical density was recorded at 290nm. Superoxide dismutase (EC 1.15.1.1) was determined by following method of Giannopolitis and Ries (1977). The absorbance was recorded at 560 nm. Bradford (1976) method was adopted to access total soluble protein contents in all treated and non-treated wheat leaf samples. 0.1µL of enzyme extract was mixed with freshly prepared G-250 Coomassie Brilliant Blue dye (1.9mL). G250 Coomassie Brilliant Blue was prepared according to standard protocol. The absorbance was recorded at 595nm.

HPLC determination of phenolic compounds: Phenolics were extracted by using a slightly modified method of Pak-Dek *et al.,* (2011). Fine Powder of dry wheat leaves were extracted in 100% HPLC grade methanol (1:10) and filtered through 0.45 µm cellulose acetate filter (EMD Millipore, Billerica, MA, USA). An aqueous suspension of the extract was then prepared with double distilled water and adjusted to pH 2 with 6 M HCl, the resulting solution was placed at 100 °C for 1h. All of the standards for phenolics were filtered through 0.451 mm syringe membrane filter (Type Millipore) and sonicated for 15 min in a Micro clean 109 bath prior to analyze by HPLC. Phenolics were analyzed using Gradient HPLC (Shimadzu, Japan) having LC-10AT, SCTL 10A system controller, SPD-10AR UV-VIS detector at 280 nm with C18 stationary column (Shim-Pack CLC-ODS). Elution was done for 60 min with a flow rate of 1 ml/min in a gradient system of two mobile phases A (H_2O_2: AA-94:6, pH 2.27), B (ACN 100%).

Statistical analysis

Research findings were analyzed using the statistical software for social sciences (SPSS; version 21.0; SPSS Inc., USA). Three-way analysis of variance was calculated and means between the treatments was compared by post hoc Duncan's multiple range test with least significance difference of $p \leq 0.05$.

Chemical and reagents: All chemicals were of analytical grade (Sigma- Aldrich). Deionized water was used throughout. The following phenolic compounds were purchased from Sigma to be used as internal standard (IS): Quercetin, Gallic acid, Caffeic acid, p-hydroxybenzoic acid, benzoic acid, Vanillic acid, cinnamic acid, syringic acid, p-coumaric acid, m-coumaric acid, ferulic acid, sinapic acid, chlorogenic acid.

Results and Discussion

Phenolics act as secondary metabolite, antioxidant, immune enhancer and hormone modulator and its activity further enhanced under stress by exogenous application of synthetic and natural chemical.

Response of MLE and AsA against oxidants: The increasing cadmium concentration in sand medium significantly ($p \leq 0.00$) enhanced H_2O_2 contents (40-67%) at both stages of wheat (Table 1, Fig. 1a). Reactive oxygen species (ROS) enhanced in a parallel fashion with increasing Cd concentration, which is further aided by its long term exposure at boot stage (Fig. 1a). Petrov and Breusegem (2012) suggested that negative consequence of H_2O_2 is dependent on concentration of stress in the immanent vicinity of plant. Hydrogen peroxide (H_2O_2) elicited innate mechanism of wheat and mediated immediate response by accumulating antioxidant molecules under mild Cd stress (Sharma *et al.,* 2012). Both foliar spray (AsA and MLE) considerably reduced effect of H_2O_2 (10-32%). Cd effect is very slightly mitigated by both elicitors used particularly under 1000 µmol/L Cd stress at boot stages (Fig. 1a). According to Ye *et al.,* (2012), ascorbic acid can directly react with ROS and also serve as substrate for ascorbate peroxidase (APX) to mitigate harmful effects of Cd. Afzal *et al.,* (2014) also proposed that *M. oleifera* leaves, might obstruct arsenic-mediated signaling pathway or scavenge free radicals in rice through its antioxidant property. The presence of quercetin in moringa leaves may obstract oxidative demage in plants and same was reported by Shin *et al.,* (2010). However, a deep scientific study of MLE at molecular level is required to elucidate significance of individual substance to detoxify Cd in wheat. This increase in H_2O_2 resulted in lipid peroxidation and exhibited increased malondialdehyde (MDA) contents (Fig. 1b). MDA contents significantly ($p \leq 0.000$) increased (58-74%) with Cd accumulation in rhizosphere at both stages (Table 1). Boot stage showed higher accumulation of MDA contents with 500 µmol/L (63%) and 1000 µmol/L Cd (74%) which may be a consequence of higher rate of breaking unsaturated fatty acids under prolonged exposure to Cd (Fig. 1b). Foliar treatments of MLE and AsA significantly ($p \leq 0.000$) decreased MDA contents (20-30%) and mitigated the Cd toxicity up to a certain limit (500 µmol/L) in wheat and the further increase in Cd concentration showed no appreciable recovery response with applied elicitors (Table 1; Fig. 1b). MLE treatment found to be most effective at tillering stage and same findings was also reported previously (Maryam *et al.,* 2016). The decrease in lipid peroxidation by MLE may be attributed by avoiding the fluidity and maintaining membrane integrity under stress (Hanafey *et al.,* 2017; Jungklang & Songklanakarin, 2012).

Antioxidant response with MLE and AsA: On the other hand, Cd toxicity may initiate array of enzymatic and non-enzymatic antioxidants responses in wheat especially phenolic synthesis that serve as the first-line defense mechanism in wheat. Current data regarding antioxidant, presented a strong relevance for enhancement of enzymatic antioxidants (Fig. 2). Superoxide dismutase (SOD) activity decreased significantly ($p \leq 0.001$) with Cd (Table 1; Fig. 2a). However, SOD activity enhanced with the foliar application of MLE, 68 and 25% at tillering while 63 and 32% at boot stage with 500 µmol/L and 1000 µmol/L Cd respectively (Fig. 2a). Khan *et al.,* (2007) reported that foliarly applied AsA enhanced SOD activity under stress in wheat. This represented that MLE and AsA mitigated Cd toxicity by sensing it at the membrane level of plant and enhanced SOD concentration (Fig. 2a). Guaiacol peroxidase (GPX) activity affected severely with 1000 µmol/L Cd, 60% at tillering and 90% at boot stage (Fig. 2b). A drastic reduction ($p \leq 0.000$) in GPX activity was observed at both stages of wheat with Cd (Fig. 2b). However, foliar application of moringa and ascorbic acid alleviated its toxicity to some extent and enhanced GPX activity 9-24% at tillering and 20-74% at boot stage against Cd toxicity. Parveen *et al.,* (2016) also reported the same decreasing trend of GPX under Cd stress. Cd significantly affected catalase (CAT) and ascorbate peroxidase (APX) activity at both stages of wheat (Table 1). Catalase activity enhanced significantly ($p \leq 0.001$) with both exogenous applications under 500 µmol/L Cd and showed tolerance mechanism but negatively affected antioxidant pool with highest Cd concentration (Fig. 2c). Catalase activity enhanced under both Cd levels with AsA and MLE at tillering stage (Fig. 1c). Ascorbate peroxidase (APX) activity significantly ($p \leq 0.000$) enhanced with the foliar application of MLE (60%) followed by ascorbic acid (32%) in non-stressed wheat plants at tillering stage (Fig. 2d). The activities of APX and CAT increased significantly in bread wheat under Cd stress (Muhammad *et al*., 2018) while Rady *et al.,* (2015) reported that Cd caused a decrease in CAT activity in wheat. The highest level of Cd stress significantly decreased APX concentration at boot (>100%) and tillering stage (54%). These enzymatic antioxidants can avert Cd toxicity in wheat (Wu *at al*., 2016). Present work strongly suggested that enzymatic antioxidants reduced significantly with 1000 µmol/L Cd but foliar application of MLE and AsA enhanced antioxidant activities under 500 µmol/L. AsA is an important source for scavenging ROS and enhanced the transcript level of superoxide dismutase-catalase, peroxidase and ascorbate peroxidase (Xu *et al.,* 2008). Antioxidant properties of the *Moringa oleifera* leaf aqueous extract was also tested by Sreelatha & Padma

(2009) and found to be very effective for scavenging reactive oxygen species compared to reference antioxidants. Elevated level of Cd caused significant reduction (143%) in total soluble protein (TSP) contents at tillering stage of wheat (Table 1; Fig. 2e). However, foliar treatment of MLE and AsA considerably improved status of TSP under Cd stress at both stages. With special reference to boot stage, TSP activity enhanced upto 53-64% with MLE. A radical decline in TSP pool without foliar spray showed sensitivity of wheat towards cadmium stress and significance of MLE and AsA spray. It has already been proven that numerous stress related proteins particularly APX, SOD, POD, CAT and total soluble proteins get activated in response to oxidative damage caused by Cd stress in wheat. These antioxidants are able to detoxify harmful metabolites (Edwards *et al*., 2000). Additionally, use of environmental friendly techniques like MLE supports this mechanism of Cd detoxification.

Table 1. Mean Square analysis of variance of hydrogen peroxidase (H_2O_2), malondialdehyde (MDA), superoxide dismutase (SOD), guaiacol peroxidase (GPX), catalase (CAT), ascorbate peroxidase (APX) and total soluble protein (TSP) contents with exogenous application of ascorbic acid (AsA) and Moringa leaf extract (MLE) under cadmium stress in wheat.

Source	Dependent variable	DF	Mean square
Cd	H_2O_2	2	1.045***
	MDA	2	1139.538***
	SOD	2	55.324*
	GPX	2	0.212*
	CAT	2	3.695***
	APX	2	2.014**
	TSP	2	550.19***
Foliar Treatment (MLE & AsA)	H_2O_2	2	0.399***
	MDA	2	553.215***
	SOD	2	122.224**
	CAT	2	3.698***
	GPX	2	0.147**
	APX	2	1.429***
	TSP	2	191.67**
Stages	H_2O_2	1	.061**
	MDA	1	607.871*
	SOD	1	5.448***
	GPX	1	0.018***
	CAT	1	0.943***
	APX	1	0.655***
	TSP	1	94.18***
Cd × Foliar Treatment	H_2O_2	4	0.115***
	MDA	4	105.790***
	SOD	4	7.274***
	GPX	4	0.033***
	CAT	4	0.236***
	APX	4	0.080***
	TSP	4	11.75***
Cd × Stages	H_2O_2	2	0.093***
	MDA	2	236.512**
	SOD	2	2.503***
	GPX	2	0.004***
	CAT	2	0.085**
	APX	2	0.186***
	TSP	2	8.83***
Cd × Foliar Treatment × Stages	H_2O_2	4	0.017**
	MDA	4	26.749***
	SOD	4	2.607***
	GPX	4	0.005**
	CAT	4	0.057**
	APX	4	0.048***
	TSP	4	3.69**

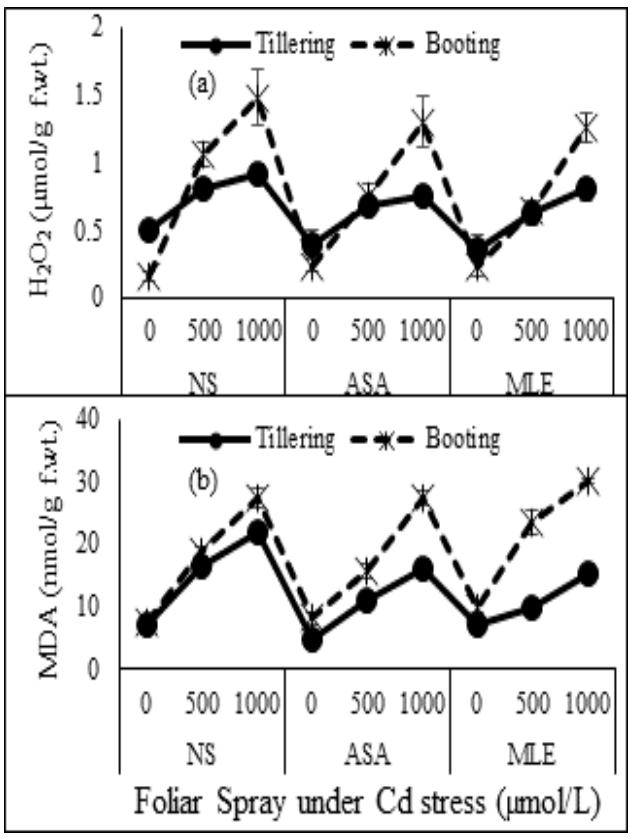

Fig. 1(a-b). Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) response in wheat with and without foliar application of MLE and AsA against Cd (± SE).

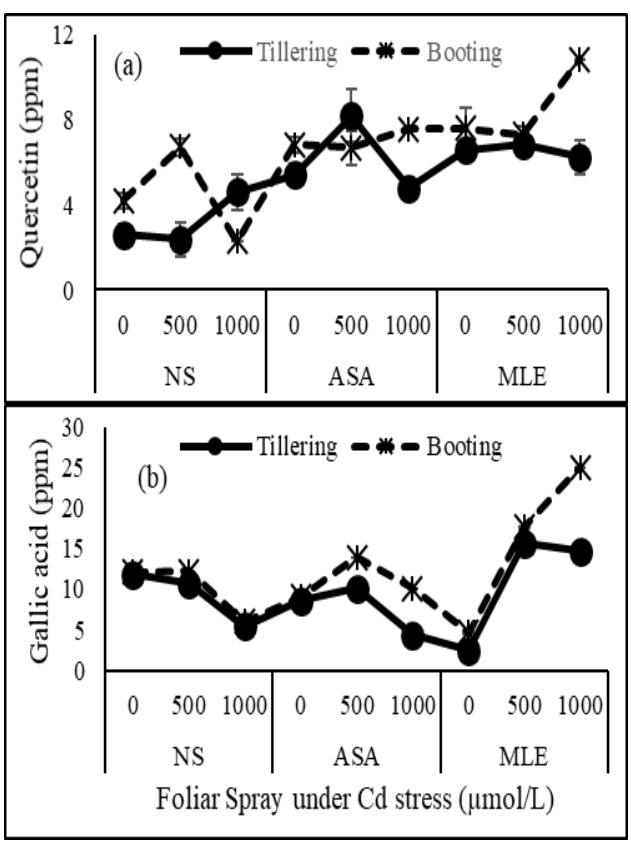

Fig. 3. Quercetin (a) and gallic acid (b) detected by HPLC in wheat with and without foliar application of MLE and AsA against Cd (± SE).

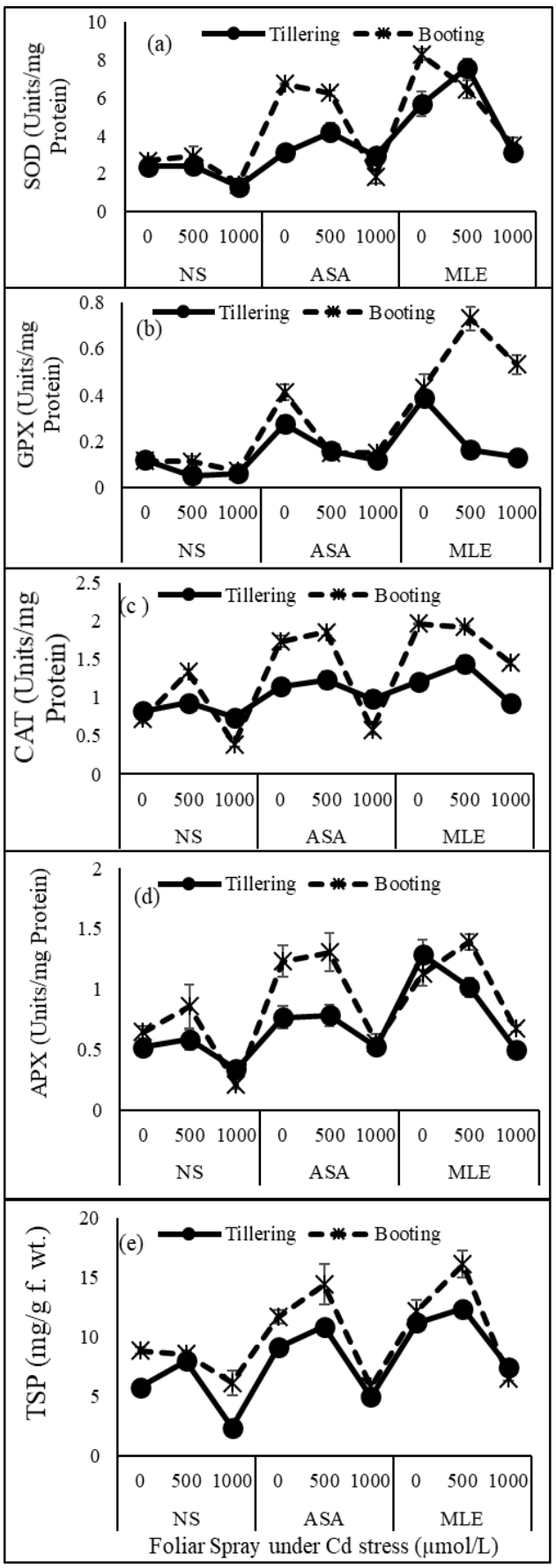

Fig. 2(a-d). Superoxide dismutase (SOD), guaiacol peroxidase (GPX), catalase (CAT) and ascorbate peroxidase (APX) response in wheat with and without foliar application of MLE and AsA against Cd (± SE).

Table 2. Effect of cadmium on phenolic synthesis after exogenous treatment of AsA and and MLE at tillering stage of wheat (Nd = Not detected).

Name of component (ppm)	Retention time	Control			500 µmol/L			1000 µmol/L		
		NS	AsA	MLE	NS	AsA	MLE	NS	AsA	MLE
Quercetin	3.247	3.476 ± 1.0	5.27 ± 1.02	6.23 ± 0.504	2.416 ± 1.62	8.07±2.52	6.868 ± 0.82	4.604 ± 0.83	4.74 ± 0.39	6.232 ± 0.81
Gallic acid	5.273	4.792 ± 0.05	3.34 ± 0.141	12.86 ± 0.01	0.964 ± 0.59	4.06±0.01	5.584 ± 0.75	2.16 ± 0.13	39.2 ± 0.01	1.728 ± 0.63
Caffeic acid	12.6	Nd	Nd	Nd	0.91 ± 0.041	2.06±0.05	Nd	Nd	19.82 ± 0.231	55.6 ± 0.056
Vanillic acid	13.047	Nd	Nd	Nd	1.212 ± 0.08	Nd	0.916 ± 0.25	Nd	Nd	Nd
Benzoic acid	14.753	Nd	Nd	15.34 ± 0.06	Nd	48.8±0.057	Nd	Nd	Nd	Nd
Chlorogenic acid	15.62	Nd	1.5±0.071	15.25 ± 0.04	6.14 ± 0.093	Nd	35.89 ± 0.06	Nd	18.12 ± 0.09	2.74 ± 0.17
Syringic acid	16.22	0.17 ± 0.03	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
p-Coumeric acid	17.14	Nd	Nd	3.872 ± 0.05	Nd	Nd	2.116 ± 0.059	1.79 ± 0.06	Nd	3.15 ± 0.05
m-Coumeric acid	20.167	4.26 ± 0.22	Nd	2.82 ± 0.14	0.98 ± 0.09	Nd	0.664 ± 0.067	Nd	Nd	Nd
Sinapic Acid	26.26	Nd	Nd	Nd	1.83 ± 0.071	Nd	Nd	0.46 ± 0.05	5.90 ± 0.09	Nd

Table 3. Effect of cadmium on phenolic synthesis after exogenous treatment of AsA and MLE at boot stage of wheat (Nd = Not detected).

Name of component (ppm)	Retention time	Control			500 µmol/L			1000 µmol/L		
		NS	AsA	MLE	NS	AsA	MLE	NS	AsA	MLE
Quercetin	3.247	4.19 ± 0.36	6.83 ± 0.34	7.27 ± 0.84	6.78 ± 1.66	6.63 ± 0.78	7.60 ± 0.04	2.26 ± 0.01	7.55 ± 0.01	10.83 ± 0.00
Gallic acid	5.273	12.09 ± 0.171	9.08 ± 0.22	4.67 ± 0.47	2.95 ± 0.02	4.90 ± 0.01	7.06 ± 0.041	2.34 ± 0.04	10 ± 0.02	2.57 ± 0.02
Caffeic acid	12.6	Nd	Nd	Nd	10.10 ± 0.07	Nd	0.59 ± 0.091	Nd	Nd	6.71 ± 0.04
Vanillic acid	13.047	Nd	Nd	Nd	Nd	32.02 ± 0.26	50.29 ± 0.02	Nd	Nd	2.008
Benzoic acid	14.753	Nd	Nd	5.09 ± 0.27	Nd	Nd	Nd	Nd	Nd	32.48 ± O.08
Chlorogenic acid	15.62	3.91 ± 0.056	35.86 ± 0.56	17.9 ± 0.62	Nd	8.18 ± 0.02	7.52 ± 0.04	Nd	Nd	Nd
Syringic acid	16.22	0.17 ± 0.029	Nd	Nd	Nd	Nd	Nd	4.48 ± 0.06	19.26 ± 0.07	Nd
p-Coumeric acid	17.14	Nd	Nd	3.87 ± 0.05	4.56 ± 0.09	Nd	Nd	Nd	Nd	1.13 ± 0.07
m-Coumeric acid	20.167	9.92 ± 0.201	Nd	3.28 ± 0.14	Nd	Nd		1.86 ± 0.10	8.86 ± 0.08	1.83 ± 0.72
Sinapic acid	26.26	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd

Modulation of phenolic synthesis: Phenolic compounds expressed differentially, only a few compounds are presented to be strongly involved in Cd tolerant mechanism in wheat. Current data suggested that phenolic compounds showed significant variation with increasing Cd toxicity at both stages of wheat (Tables 2-3). At boot stage of wheat, quercetin activity enhanced with foliar application of MLE (10.83 ± 0.00) compared to no spray plants (2.26 ± 0.04) under 1000 µmol/L Cd stress (Table 3; Fig 2a). Muhammad *et al*., (2010) also reported variation in lipid peroxidation and phenolic contents at different growth stages of wheat (vegetative, booting and reproductive). Maximum quercetin concentration was observed with MLE (6.86±0.82) to mitigate Cd toxicity at tillering stage (Fig. 3a). Gallic acid was another promising phenolic compound seems to be involved in conferring tolerance in plants with exogenous application of MLE and AsA. Gallic acid concentration was particularly enhanced at tillering stage with AsA (39.2 ± 0.01) under 1000 µmol/L Cd (Table 2). The chlorogenic (35.89±0.08) and caffeic acid (55.6±0.05) concentration was mounted with foliar application of MLE against 500 µmol/L and 1000 µmol/L Cd respectively (Table 2). At boot stage, vanillic acid concentration raised with MLE (50.29±0.02) and AsA (32.02±0.26) against 500 µmol/L Cd toxicity (Table 3). Sinapic acid, benzoic acid, *p*-coumeric acid, *m*-coumeric acid expressed differentially with and without Cd stress, showed weak response towards foliar application of MLE and AsA (Tables 2-3). According to Gao *et al.,* (2016) transcript level of phenolic compound also increased immediately upon receiving stress signals through shikimate pathway. Gallic acid, chlorogenic acid, quercetin and kaempferol are the most abundant phenolic compounds identified in the leaf extract of moringa leaf extract (Nikkon *et al.,* 2015). As the current data suggested that quercetin being a dominant phenolic compound, increased with foliar application of AsA and MLE compared to control/non-stressed wheat samples. A substantial increase in the quercetin level was also recommended in the roots of wheat under stress (Gondor *et al.,* 2016). Khursheda *et al.,* (2019) also demonstrated that high concentration of quercetin also confers tolerance against salinity. Manquian-Cerda *et al.,* (2016) also correlated chlorogenic activity with enhanced Cd tolerance.

Conclusion

Taken as a whole, the investigation of current study demonstrated that Cd treatment particularly at high dose induce oxidative damage and lipid peroxidation in wheat. However, the foliar application of MLE and AsA enhanced antioxidant potential of wheat under moderate level of stress (5000 µmol/L). Moreover, some of the phenolic compounds especially chlorogenic, caffeic acid and vanilic acid concentration increased multifold with exogenously applied MLE and AsA under Cd stress. Quercetin and gallic acid were the major phenolic compounds to create tolerance in wheat against Cd with the involvement of MLE and AsA.

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