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Action of cadmium toxicity on growth, physiological activities and subcellular components of watercress (Eruca sativa L.) plant: The protective role of salicylic acid.

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ABSTRACT

Seed germination, seedling growth, leaf morphology, physiological alterations and cell ultrastructure of watercress (Eruca sativa L.) plant under cadmium (Cd) and salicylic acid (SA) treatments were studied. Cd concentrations (0.1 and 0.5 mM), decreased seed germination, shoot and root lengths, but with 1 mM Cd was inhibited. Cd-treated watercress displayed leaf chlorosis. Photosynthetic pigment contents of Cd-treated plants were declined with increasing Cd treatments. Malondialdehyde (MDA) and total phenolic contents increased while soluble proteins decreased in response to Cd treatments. Electron microscopic observations of Cd-treated plants revealed disorganization in the internal structure of chloroplasts and mitochondria. The lamellae and stroma thylakoids of chloroplasts were degenerated. An increase of plastoglobuli number within chloroplasts was observed in Cd-treated leaves. Addition of SA (50 and 100 µM) to 0.5 mM Cd improved seed germination and seedling shoot length but reduced root length of watercress compared to that only treated with Cd, however, SA was not able to alleviate seed germination at 1 mM Cd. Spraying of 50 and 100 µM SA three days before Cd-treated plants reduced chlorosis and increased pigment contents of leaves compared to that only treated with Cd. SA caused a decrease in MDA and soluble proteins contents, and increased total phenolic compounds of Cd treated plants. On the level of ultrastructure, SA-treated plants protected grana organization and reduced plastoglobuli number of chloroplast against Cd effect. The results correlated to oxidative stress, such as chlorosis, decreased photosynthetic pigment content, increased lipid peroxidation, and damage of cell organelles in response to Cd effect were resulted. SA pretreatment reduced the negative effect of Cd on plant parameters mentioned above associated with the decrease of oxidative stress.

Keywords: Cadmium, Metabolites, Oxidative stress, Salicylic acid, Ultrastructure, Watercress

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INTRODUCTION

Due to increasing anthropogenic activities in various industrial applications and to heavy metals mobilization through rocks extraction, the contamination of agricultural soil and waters by heavy metals has become a considerate problem in the world. Moreover, heavy metals are non-biodegradable; thus, they accumulate in the tissues of living organisms and cause toxic effects [1-4]. Cadmium is non-essential for plants function activities [5] and commonly added to the agricultural soil through addition of phosphoric fertilizers to farming soils and other different sources such as pesticides and mining, and accumulate within plants [6, 7]. Cd is water soluble and so quickly adsorbed in plant tissues. Its accumulation in crop plants causes a severe threat to human health through food chains [8, 9]. The main sources of Cd uptake in human are via eating food stuffs that have Cd [10, 11]. The average of Cd concentration in non-polluted soil is between 0.04–0.32 mM, whereas the moderate and highly polluted soil in between 0.32–1.00 mM [12-14]. Cd is toxic to plants at 5 to 10 mg kg^{-1} in the leaf dry matter [15]. Cd toxicity is resulted from increasing reactive oxygen species (ROS), which caused oxidative stress [16, 17]. Under effect of extreme stress conditions, the antioxidant ability may not be enough to reduce the negative effect of oxidative injury that resulted from the over production of free radicals. Accumulation of Cd in higher plants caused inhibition of plant growth, water uptake, disturbance of nutrient uptake, leaf chlorosis, inhibits metabolic and photosynthetic activity, and alteration of cell organelles ultrastructure [18-22].

Salicylic acid (SA) is a signal molecule in plants and involved in specific responses to environmental stresses [23-25]. SA alleviates stress effects of temperature and heavy metals on plants [26-29]. Moreover, SA modified plant responses to salt and osmotic stresses [30, 31] herbicides [32], and pathogens [33, 34]. Decline Cd accumulation in Kentucky bluegrass due to pretreatment of SA was concomitant with increasing chlorophylls, growth and nutrient elements, and reducing malondialdehyde and H_2O_2 contents [35]. Also, SA pretreatment alleviates Cd injury in wheat and barley [36, 37]. Moreover, SA alleviated growth inhibition caused by Cd toxicity in various plant species [38, 39]. CO_2 fixation rate was partially overwhelmed in SA-pretreated plants [40]. Furthermore, Cd-induced stress responses were markedly reversed by SA post-treatment [41]. Although there have been many studies on the level of cellular, molecular and biochemical of plants affected Cd toxicity, however, to own knowledge, only limited studies has been carried out on vegetable plants as watercress (Eruca sativa L.). Thus, the objective of this study was to identify the seed germination, ultrastructural and physiological changes resulting from the application of cadmium on watercress plants. Also, the study was to identify the role of SA for alleviation the negative effect of Cd on watercress.

MATERIALS AND METHODS

Seed surface sterilization and treatments

Seeds of watercress (Eruca sativa L.) were first immersed in 70% ethanol for 3 min and then in 0.1% $HgCl_2$ for 5 min. The seeds were then washed for five times with distilled water to remove the sterilized materials. The sterilized seeds (20 seeds) were spread over sterilized Petri-dishes lined with filter paper. The tested solutions were comprised of control and three Cd concentrations (0.1, 0.5 and 1 mM). Three replicates for each treatment were used. The tested solutions were applied to each Petri-dish. On day 5, the germinated seedlings were transferred to another set of sterilized Petri-dishes with renewed of the tested solutions. In parallel, the interaction of two SA concentrations (50 and 100 µM) with Cd concentrations was performed. On day 10, three replicates for germination assay, hypocotyls and radical lengths were measured. Petri-dishes were placed in an incubator at 25 ± 2°C. Control was provided with distilled water free Cd and SA. Germination index of the different treatments were calculated using the formula: G.I. = (no. of seeds germinated/total no. of seeds) ×100. The length of root and shoot of seedling were estimated under control and various treatments.

Soil culture experiment

In the second experiment, watercress seeds were surface sterilized as mentioned above. Seeds were sown in clean plastic pots containing 500 g soil mixture composed of sand and soil at 1:1 v/v ratio. Based on the seed germination results, the concentration of Cd and SA were chosen for soil experiment. After one week from the seed emergence, watercress plants were distributed into seven groups as follows. Group 1, plants were used as control; groups, 2 and 3 plants were treated with 0.5 and 1 mM Cd, respectively; groups, 4 and 5 plants were treated with 0.5 mM Cd + 50 µM SA and 0.5 mM Cd + 100 µM SA, respectively; groups, 6 and 7,

plants were treated with 1.0 mM Cd + 50 µM SA and 1.0 mM Cd + 100 µM SA, respectively. Plants were treated with the tested solutions for four weeks in open field. Shoot samples of the control and treated plants were collected separately in three replicates. The morphological, physiological parameters and cell ultrastructure of treated and untreated plants were studied.

Determination of photosynthetic pigments

Contents of chlorophyll a (Chl a), chlorophyll b (Chl b) and total carotenoids (Car) of watercress leaves were spectrophotometrically (Jenway 6300 spectrophotometer, UK) determined according to Lichtenthaler [42]. The photosynthetic pigment content was extracted from a known fresh weight of leaves in 85% (v/v) aqueous acetone. The extract was centrifuged at 4,000 × g for 10 min. The absorbance was measured against a blank of pure 85% aqueous acetone at three wavelengths 663, 647 and 470 nm according the following equations:

$$\text{Chl a} = 12.25\ A663 - 2.79\ A647$$
$$\text{Chl b} = 21.50\ A647 - 5.10\ A663$$
$$\text{Car} = (1000 \times A470 - 1.82 \times \text{Chl a} - 95.15 \times \text{Chl b})/225$$

The pigment contents (Chl a, Chl b and carotenoids) were calculated as mg g^{-1} FW.

Determination of malondialdehyde

Malondialdehyde (MDA) content was determined as an indication of leaf lipid peroxidation according to Hernández and Almansa [43]. Fresh leaf samples (500 mg) were homogenized in 5 mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000 × g for 20 min at 4°C. One mL aliquot of the supernatant was mixed with 3 mL of 0.5% thiobarbituric acid (TBA) prepared in 20% TCA and incubated at 90°C for 20 min. After stopping the reaction in an ice bath, samples were centrifuged at 10,000 × g for 5 min. The supernatant absorbance at 532 nm was then measured. After subtracting the non-specific absorbance at 600 nm, MDA concentration was determined using the extinction coefficient 155 $mM^{-1}cm^{-1}$.

Determination of soluble protein content

Soluble protein content of leaves was determined according to Lowry et al. [44] Leaf samples (0.1 g dry weight) were extracted in 10 mL distilled water for 2 h at 90°C. The extracts were centrifuged and the supernatants were collected. One mL of extract was added to 5 mL of alkaline reagent (50 mL 2% Na_2CO_3 prepared in 0.1 N NaOH and 1 mL 0.5% $CuSO_4.5H_2O$ prepared in 1% sodium potassium tartarate) and mixed thoroughly then allowed to stand for 10 min. A total of 0.5 mL of Folin-Ciocalteu reagent diluted 1:2 (v/v) was then added and mixed immediately. After 30 min, the extinction against appropriate blank was measured at 700 nm. Bovine serum albumin was used as a standard. Protein contents were expressed as mg g^{-1} DW.

Determination of total phenolic compounds

Total phenolics were measured according to Dai et al. [45]. Twenty five µL of the extract was mixed with 110 µL Folin-Ciocalteu reagent, 200 µL 20% sodium carbonate and 1.9 mL distilled water, and placed at 60°C for 30 min. Optical density was measured with a spectrophotometer at 750 nm. A standard curve was constructed with different concentrations of gallic acid. The results were expressed as µg of gallic acid g^{-1} FW.

Ultrastructure studies

For ultrastructure studies, fresh leaf samples (1-2mm) were fixed in 3% glutaraldehyde prepared in 0.05 M phosphate buffer (pH 7), for 3 h. Samples were rinsed several times in 0.05 M phosphate buffer and then were post fixed with 1% OsO4 in 0.05 M phosphate buffer for 2 h. Samples were rinsed several times with 0.05 phosphate buffer and then dehydrated in a gradient ethanol series, and embedded in epon 812 [46]. Ultrathin sections (60-70 nm thick) were stained with uranyl acetate and lead citrate. Specimens were viewed with a Jeol-1011 transmission electron microscope (Unit of Electron Microscopy at Taif University, Saudi Arabia).

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Statistical analysis

The results were tested for significance by using one-way of variance (ANOVA) test. Means were compared by least significant differences (LSD) test at levels P<0.05 and P<0.01. The statistical tests were carried out using SPSS 9.0 statistical software for Windows.

RESULTS AND DISCUSSION

Seed germination and seedling growth

Soils and water contaminated heavy metals has created a major environmental risk, leading to considerable loss in plant growth and pose serious threats to human health. Action of Cd^{+} and the interaction of Cd + SA were evaluated through watercress seed germination and seedling growth (Table 1). Severity of Cd treatments on seed germination and seedling growth (shoot and root lengths) of watercress was resulted. At 0.1 and 0.5 mM Cd, the watercress seed germination decreased by 3.8 and 89.7%, respectively, compared to the control, while it was inhibited at 1 mM Cd. Interaction of SA (50 and 100 µM) and Cd treatments (0.5 and 1 mM) increased seed germination as compared to that only treated with 0.5 and 1 mM Cd (Table 1).

Table 1: The influence of 10-day Cd and Cd + SA interactions on seed germination (%), root and shoot lengths (Cm) of watercress. The values are means (M) of three replicates ± standard deviation (SD).

*, significant at P<0.05; **, significant at P<0.01.

Treatments	Germination (%)				Root length (cm)				Shoot length (cm)			
	M	±	SD	%	M	±	SD	%	M	±	SD	%
Control	97.3	±	1.52	100	2.63	±	0.51	100	4.15	±	0.30	100
0.1 mM Cd	93.6*	±	1.52	96.2	1.63**	±	0.32	62	3.55**	±	0.18	85.5
0.5 mM Cd	10.0**	±	2.00	10.3	0.53**	±	0.15	20.1	1.12**	±	0.03	27.0
1.0 mM Cd	00.0**	±	0.0	00.	0.00**	±	0.00	0.0	0.00**	±	0.00	0.0
0.5 mM Cd + 50 µM SA	17.6**	±	1.52	18.1	0.35**	±	0.05	13.3	1.32**	±	0.10	31.8
0.5 mM Cd + 100 µM SA	14.7**	±	2.51	15.1	0.42**	±	0.06	16.9	1.35**	±	0.10	32.5
1.0 mM Cd + 50 µM SA	00.0**	±	0.0	00.0	0.00**	±	0.00	0.0	0.00**	±	0.00	0.0
1.0 mM Cd + 100 µM SA	00.0**	±	0.0	00.0	0.00**	±	0.00	0.0	0.00**	±	0.00	0.0

In details, the addition of 50 and 100 µM SA to 0.5 mM Cd increased seed germination by 7.8 and 4.8 % as compared to that only treated with 0.5 mM Cd. In contrast, SA (50 and 100 µM) did not alleviate the inhibition of seed germination at 1 mM Cd. Seedling shoot and root lengths of watercress were reduced in response to 0.1 and 0.5 mM Cd. Following the mentioned Cd level treatments, the root length at 0.1 and 0.5 mM Cd decreased by 38 and 79.8%, respectively, compared to the control, while root growth was inhibited at 1 mM Cd (Table 1). Similar action of Cd was detected in shoot length at 0.1 and 0.5 mM Cd, the decrease was 14.5, 73 and 100%, respectively, as compared to the control. SA + Cd interactions caused more reduction in root length compared to that only treated with Cd. On the other hand, the shoot length under effect of SA + Cd was increased compared to that only treated with Cd. The results revealed that the root growth was more affected than shoot under Cd effect (Table 1). SA treatment reduces the negative action of Cd on rice seed germination and root length [47] and on pea seedlings [48]. These studies support the present results, which revealed that the negative action caused by Cd was alleviated by exogenous application of SA.

Leaf symptoms

Untreated leaves of watercress plants did not show any symptoms of damage, such as chlorosis or necrosis, and they were dark green (Fig. 1A). Leaf chlorosis was resulted due to Cd concentration treatments (Fig. 1B and 1C).The chlorosis symptoms symmetrically spread in the whole leaves. Appearance of chlorosis on watercress leaves reflected injures and oxidative stress. Cd-treated plants caused chlorosis, water uptake imbalance, stomatal closure, delay of growth and development [49, 50]. The effect of 50 and 100 µM SA spraying three days before treatments of 0.5 and 1 mM Cd (Fig. 1D and 1E, respectively) reduced the appearance of chlorosis compared to that only treated with 0.5 mM Cd (Fig. 1B). Figure (1G and 1F) showed the

effect of 50 and 100 µM SA three days before 1 mM Cd treated plants, respectively. SA did not alleviate the oxidative stress as indicated by chlorosis that appeared on leaves at 1 mM Cd (Fig. 1 F and 1G).

Photosynthetic pigments

The content of photosynthetic pigments (Chl a, Chl b, and Car) of water cress leaves were significantly declined in response to increasing Cd applications compared to the control (Table 2). In this respect, Chl a content showed 53.2 and 71.1% reduction in 0.5 and 1 mM Cd treated plants, while the reduction in Chl b content was 52.3 and 69.7%, and for carotenoid content, the reduction was 48.8 and 63.4% in 0.5 and 1 mM Cd treated plants, respectively (Table 2). The present study showed that the Chl a content decreased more than Chl b content in response to Cd treatments, and it caused a decrease in the Chl a/b ratio suggesting that the Chl a was more sensitive than Chl b to Cd treatments.

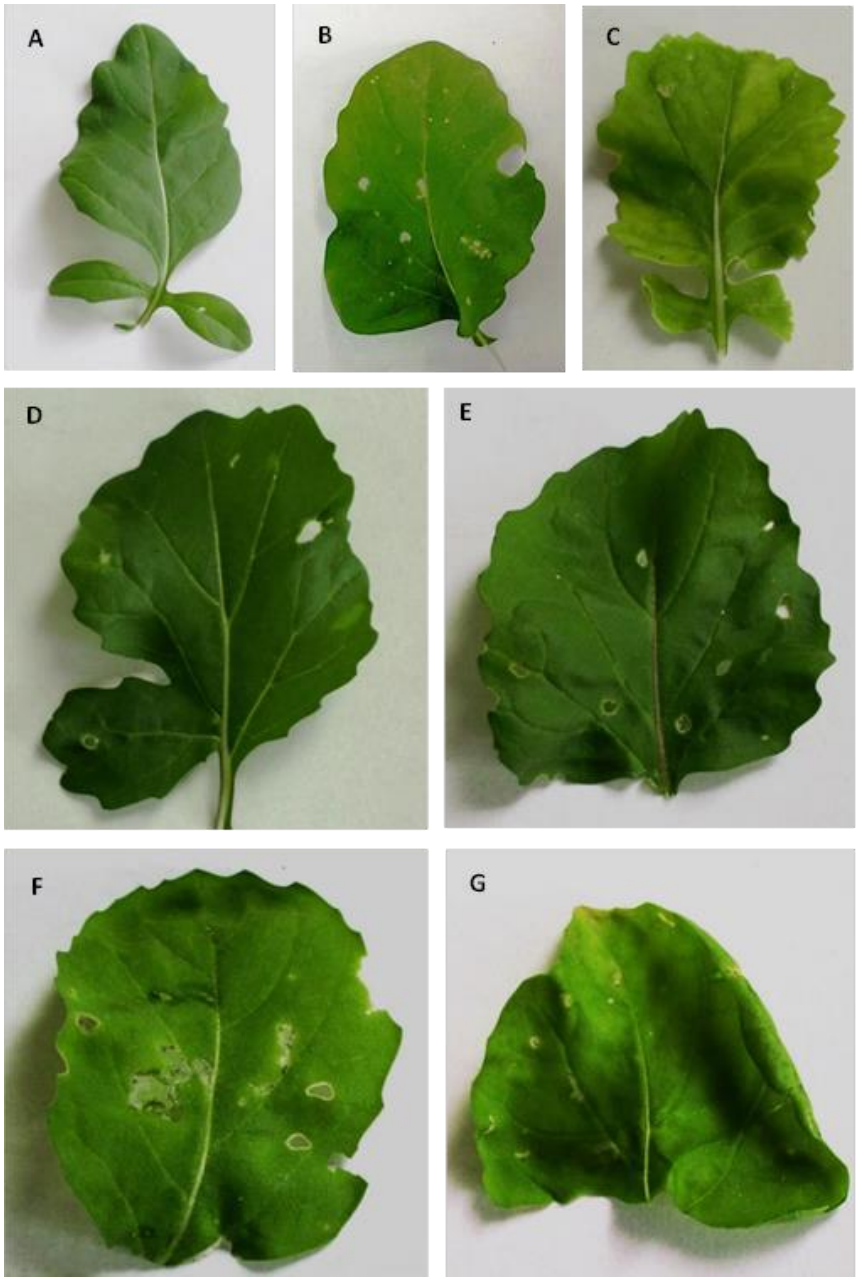

Fig 1: Light micrograph showed effect of Cd and Cd + SA treatments on leaf morphology of watercress leaves; control (A), 500 µM Cd (B), 1000 µM Cd (C), 500 µM Cd + 50 µM SA (D), 500 µM Cd + 100 µM SA (E), 1000 µM Cd + 50 µM SA (F) and 1000 µM Cd +100 µM SA (G).

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Table 2: The influence of four weeks cadmium applications and Cd + SA interactions on photosynthetic pigments of watercress leaves. The values are means (M) of three replicates ± standard deviation (SD). *, significant at P<0.05; **, significant at P<0.01.

Treatments	Chl A				Chl B				Carotenoids				A/B ratio	Total	%
	M	±	SD	%	M	±	SD	%	M	±	SD	%			
Control	1.66	±	0.30	100	0.67	±	0.11	100	0.41	±	0.10	100	2.48	2.74	100
0.5 mM Cd	0.77**	±	0.08	46.4	0.32**	±	0.03	47.7	0.21**	±	0.03	51.2	2.41	1.30	47.4
1.0 mM Cd	0.48**	±	0.14	28.9	0.21**	±	0.03	31.3	0.15**	±	0.03	36.6	2.28	0.84	30.6
0.5 mM Cd + 50 µM SA	0.90**	±	0.17	54.2	0.35**	±	0.01	52.2	0.22**	±	0.01	53.6	2.57	1.47	53.6
0.5 mM Cd + 100 µM SA	0.92**	±	0.10	55.4	0.36**	±	0.01	53.7	0.32*	±	0.02	78.0	2.55	1.60	58.4
1.0 mM Cd + 50 µM SA	0.65**	±	0.05	39.1	0.30**	±	0.02	44.8	0.20**	±	0.02	48.8	2.16	1.15	41.9
1.0 mM Cd + 100 µM SA	0.65**	±	0.05	39.1	0.31**	±	0.08	46.3	0.21**	±	0.05	51.2	2.09	1.17	42.7

SA pretreatment (50 and 100 µM) three days before 0.5 and 1 mM Cd treated plants increased photosynthetic pigments compared to that only treated with 0.5 and 1 mM Cd, respectively. In response to 50 and 100 µM SA, the Chl a of 0.5 mM Cd treated plants increased by 18.6 and 19.4% compared to that only treated with 0.5 mM Cd, respectively, while 50 and 100 µM SA increased the Chl a of 1 mM Cd treated plants by 35.4% compared to that only treated with 1 mM Cd. For Chl b, treatment with 50 and 100 µM SA increased Chl b of 0.5 mM Cd by 9.4 and 12.5%, respectively, compared to that only treated with 0.5 mM Cd. The same doses of SA increased Chl b contents of 1 mM Cd treated plants by 42.8 and 47.65%, respectively, compared to that only treated with 1 mM Cd. With increasing SA application, the Car contents of Cd treated plants significantly increased. The carotenoid contents of plants sprayed with 50 and 100 µM SA three days before treated with 0.5 mM Cd, increased by 4.7 and 52.7% compared to that only treated with 0.5 mM Cd. Similar doses of SA increased the carotenoid contents of plants treated with 1 mM Cd by 33.3 and 40% compared to that only treated with 1 mM Cd. Cd treatments significantly decreased chlorophyll and carotenoid contents in Phaseolus vulgaris [51]. A decrease in chlorophyll and carotenoid contents, and destruction of chloroplast membrane structure due to Cd application was reported [13, 52-54]. Moreover, Cd effect caused a decrease in photosynthetic quantum yield and activity of enzymes involved in CO_2 fixation [55]. The results illustrated that the phytotoxicity of Cd correlated with the reduction of pigments, damage of chloroplast and other cell organelles was comparable to that of herbicides [56-58] heavy metals [59], pathogen [60] salt and drought [31]. In the present study, the negative action of Cd on watercress may partially result from its influence on photosynthetic pigments in plants. SA treatments improved plant status by increasing pigment contents of Cd treated watercress.

Lipid peroxidation, soluble proteins and total phenolic compounds

Lipid peroxidation is one of biochemical markers for the free radical mediated injury under various abiotic stresses [61]. The leaf MDA content significantly increased due to Cd treated and to that treated with Cd + SA compared to that of the control (Table 3). Due to 0.5 and 1 mM Cd, the MDA content of watercress leaves increased by 58.7 and 64.3 %, respectivelly, compared to that of the control. In response to 50 µM SA, the MDA content of 0.5 and 1 mM Cd increased by 43.9 and 31% respectively, compared to that of the control. While with 100 µM SA, the MDA content of 0.5 and 1 mM Cd increased by 48.1 and 52.4%, respectively, compared to that of the control.

Table 3: The influence of four weeks cadmium applications and Cd + SA interactions on MDA content of watercress leaves. The values are means (M) of three replicates ± standard deviation (SD). *, significant at P<0.05; **, significant at P<0.01.

Treatments	MDA (µmol g^{-1} FM)			
	M	±	SD	%
Control	15.27	±	2.55	100
0.5 mM Cd	24.24**	±	3.93	158.7
1.0 mM Cd	25.09**	±	1.95	164.3
0.5 mM Cd + 50 µM SA	21.98**	±	3.38	143.9
0.5 mM Cd + 100 µM SA	22.62**	±	2.32	148.1
1.0 mM Cd + 50 µM SA	20.01*	±	1.35	131.0
1.0 mM Cd + 100 µM SA	23.27**	±	2.64	152.4

The oxidation of lipid is considered as the significant oxidative stress biomarkers in stressed plants [62, 63]. Lipid peroxidation occurs as a result of attack by free radicals such as reactive oxygen species (ROS) in biological systems [64]. ROS damage all major plant cell bio-polymers, resulting in their dysfunction [65]. Presoaking of flax seeds in SA before subjected to Cd decreased phospholipid of root at seedling stage [66]. Peroxidation of lipid leads to the destruction of membranes of cell organelles and dysfunction of proteins, DNA and RNA [67, 68].

Soluble proteins (Table 4) of watercress leaves were declined in response to Cd and Cd + SA treatments. The soluble proteins content of 0.5 and 1 mM Cd decreased by 8 and 22.4%, respectively,

compared to the control. SA caused more reduction in soluble protein. Spraying of 50 and 100 µM SA to plants that only treated with 0.5 mM Cd reduced the soluble proteins contents by 7.32 and 3.34%, respectively, compared to that only treated with 0.5 mM Cd. Soluble protein content of 1 mM Cd + 50 µM SA treated plants approximately had the same value of that only treated with 1 mM Cd. In contrast, spraying of 100 µM SA increased soluble protein by 16.73% compared to that only treated with 1 mM Cd. In contrast to soluble protein content, the total phenolic compound of watercress leaves, in most cases, increased due to Cd and SA treatments (Table 4). The total phenolic content of plants treated with 0.5 or 1 mM Cd and sprayed with 50 µM SA was significantly increased compared to the control and to that only treated with Cd. Changes of plant protein with various abiotic stresses were reported [31, 58].

Table 4: The influence of four weeks cadmium applications and Cd + SA interactions on soluble protein and total phenolic contents of watercress leaves. The values are means (M) of three replicates ± standard deviation (SD). *, significant at P<0.05; **, significant at P<0.01.

Treatments	Soluble proteins (mg g^{-1} DW)				Total phenolic (µg g^{-1} DW)			
	M	±	SD	%	M	±	SD	%
Control	40.05	±	5.06	100	17.85	±	1.00	100
0.5 mM Cd	36.85	±	4.64	92.0	17.78	±	0.62	99.6
1.0 mM Cd	31.08**	±	2.63	77.6	18.67	±	2.60	104.6
0.5 mM Cd + 50 µM SA	34.15*	±	1.63	85.2	25.19**	±	1.39	141.1
0.5 mM Cd + 100 µM SA	35.62	±	1.68	88.9	18.68	±	2.31	104.6
1.0 mM Cd + 50 µM SA	31.00**	±	3.39	77.4	24.03**	±	1.57	134.6
1.0 mM Cd + 100 µM SA	36.28	±	1.08	90.6	19.66	±	2.27	110.1

Phenolic compounds are widely distributed in the plant kingdom. Several of these compounds play important physiological and ecological roles being involved in resistance to different types of stress [69]. Such responses had been recorded in plants under heavy metal stress [70]. Cadmium is highly toxic to plants, water soluble and therefore promptly adsorbed in tissues and its presence greatly influences the entire plant metabolism [12]. Heavy metals such as Cd can produce ROS directly via the Fenton and Haber–Weiss reactions, and indirectly by inhibiting antioxidant enzymes [71].The adverse effect of Cd on plant growth and development has been widely documented [72]. Pretreatment of SA declined the negative effect of cadmium on wheat plants via a decline in MDA level and electrolyte leakage. Furthermore, SA-pretreatment contributed to maintenance of growth characteristics of wheat seedlings at the level close to the control under stress conditions and to acceleration of growth recovery during post-stress period [73]. Cadmium (Cd) considers hazardous heavy metals for almost all organisms. Several biochemical and molecular mechanisms have been suggested to be responsible for Cd toxicity in plants, but the disorder of reactive oxygen species (ROS) homeostasis indeed plays an important role in the development of Cd toxicity symptoms [16, 55].

Cell Ultrastructure

Electron microscopic observations of Cd (Fig. 3A and 3B), and interactions of 1 mM Cd + 50 µM SA (Fig. 4A and 4B) , 1 mM Cd + 100 µM SA (Fig. 5A and 5B) treated watercress showed much subcellular disorder compared to that of the control plants (Fig. 2A and 2B). Chloroplasts of the control plants were lens to oval-shaped, with a typical arrangement of grana and stroma thylakoids. Nucleus and mitochondria showed normal organization in the structures.

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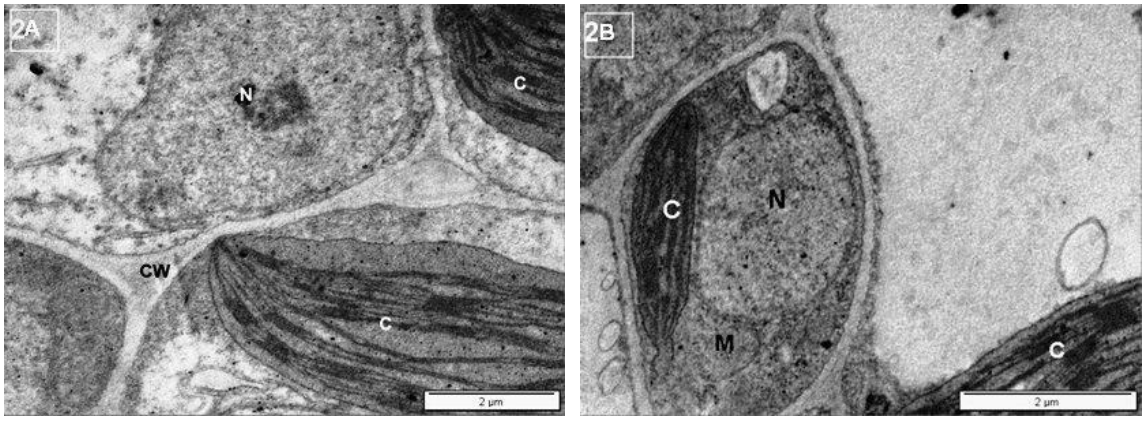

Fig 2: Electron micrograph of the control showed a portion of mesophyll cell organelles (A and B) of watercress leaves. C, chloroplast; CW, cell wall; M, mitochondria; N, Nucleus. Scale bars: 2 µm (A and B).

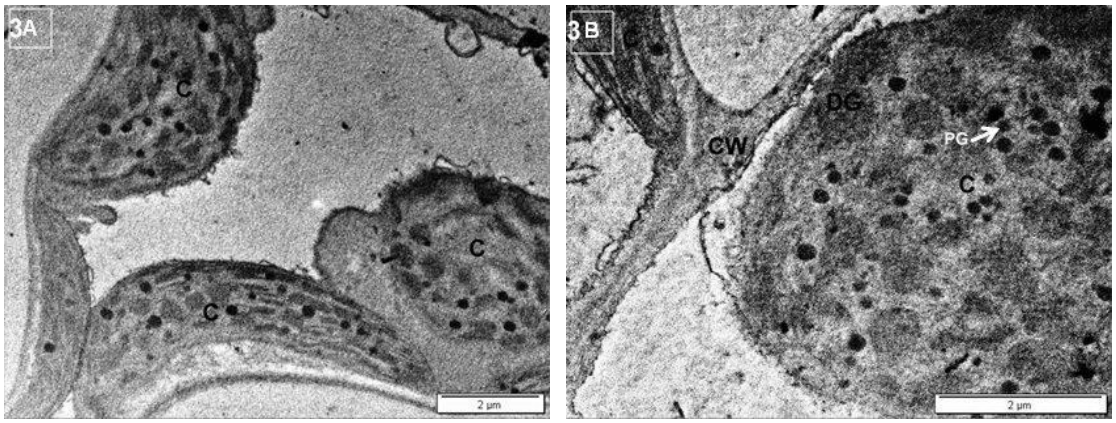

Fig. 3. Electron micrograph of 1mM Cd treated plants for four weeks showed portion of mesophyll cells (A and B) of watercress leaves. C, chloroplast; CW, cell wall; DG, degenerated granum; PG, plastoglobuli. Scale bars: 2 µm (A and B).

In contrast, chloroplasts of Cd-treated plants were strongly damaged (Fig. 3A and 3B). Degenerated grana thylakoids and disappeared of stroma lamellae were resulted in response to Cd treatments. Chloroplasts of Cd-treated plants contained many large size of plastoglobuli. Cd-treated plant appears to be ruptured the chloroplast envelope. Disturbance of membrane structure integrity and imbalanced chloroplasts lamellae formation affected Cd stress was reported [50, 74, 75].

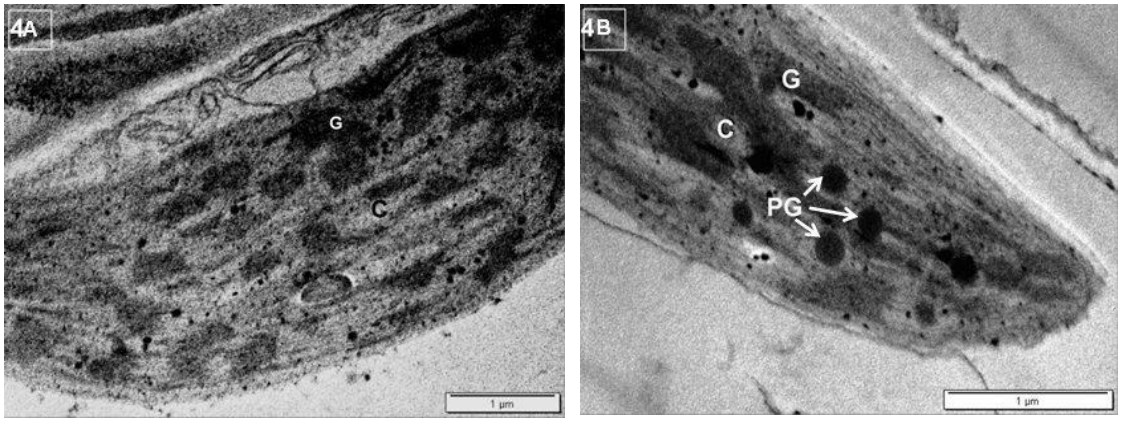

Fig 4: Electron micrograph of 1mM Cd +50 µM SA treated plants for four weeks showed portion of mesophyll cells (A and B) of watercress leaves. C, chloroplast; CW, cell wall; DG, degenerated granum; PG, plastoglobuli. Scale bars: 1 µm (A and B).

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In the present study, the alterations in chloroplasts treated with Cd were more drastic than that treated with the interactions of Cd +SA. In response to 50 and 100 µM SA, chloroplasts were partially protected from the harmful effects that resulted from 1 mM Cd-treated watercress leaves (Fig. 4A and B; Fig. 5A and B, respectively).

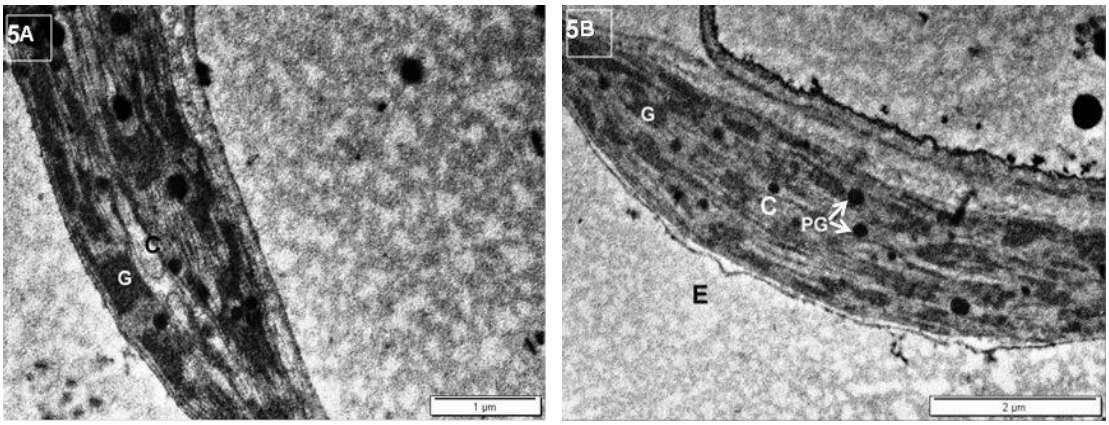

Fig 5: Electron micrograph of 1 mM Cd +100 µM SA treated plants for four weeks showed a portion of mesophyll cells (A and B) of watercress leaves. C, chloroplast; CW, cell wall; DG, degenerated granum; PG, plastoglobuli. Scale bars: 1µm (A) and 2µm (B).

SA is involved in plant protection from heavy metals [48, 66] .Chloroplast grana thylakoids and envelope, to some extent, showed normal organization in response to SA treatments compared to that only treated with 1 mM Cd. A considerable decrease in number of plastoglobuli was noticed in plant chloroplasts treated with Cd + SA compared to that only treated with Cd. These results confirmed the protective role of SA against oxidative stress of Cd.

CONCLUSION

With increasing Cd treatments, a highly significant gradual decrease in grain germination and seedling lengths occurred. At the level of plant growth, the higher Cd concentration (1 mM) disturbs the watercress leaf morphology, photosynthetic pigments and metabolites and cell ultrastructure. SA enhanced seed germination and seedling growth at low and moderate Cd-stressed plants. In light of the results obtained, it is proposed that the chlorosis observed in watercress plants could be related to degradation of chlorophylls and degeneration of chloroplast due to Cd-enhanced oxidative stress. Foliar application of SA three days before Cd associated with increasing chlorophylls and decline in MDA levels. SA partially protected grana of chloroplast. The results showed that the watercress seriously affected by Cd and the positive role of SA against Cd-stressed watercress plants.

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