Effect of cadmium stress on morpho–physiological traits in garden cress and radish in an aeroponic system

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Abstract

Cadmium is a main toxic pollutant and poses a considerable threat to human health. In other to study the effect of Cd stress on traits of garden cress and radish, an experiment was conducted based on a completely randomized design in an aeroponic system. In this study $CdCl_2$ was used at three levels (control, 3, and 6 mgl^{-1}). Seeds were cultivated in an aeroponic system and Hoagland nutrient solutions (with and without Cd) were used for irrigation. Morphological traits and cadmium accumulation were measured after 40 days. Results for garden cress showed the highest plant height (32.67 cm), leaf number (30.83), root length (14.5 cm), dry weights of roots (0.34 g) and shoots (1.52 g), fresh weights of roots (2.27 g) and shoots (11.3 g) in the control and the 6 mg l^{-1} cadmium chloride resulted in the highest cadmium accumulation in roots and shoots 2.88 and 0.11 kg/g, respectively**.** Also in radish the results showed the highest plant height (50.1 cm), leaf number (18.8), root length (26.7 cm), dry weights of roots (0.41 g) and shoots (2.04 g), fresh weights of roots (3.9 g) and shoots (27.01 g) in the control and the 6 mg l^{-1} cadmium chloride produced the highest cadmium accumulation in roots and shoots 3.3 and 0.28 kg/g, respectively**.** Findings showed that proline, total phenol, and anthocyanin contents increased under cadmium stress in garden cress and radish.

Keywords: cadmium chloride; heavy metal; soilless system; stress; vegetable

Moridian Pirdosti, Kh., Z. Movahedi and M. Rostami. 2018. 'Effect of cadmium stress on morpho-physiological traits in garden cress and radish in an aeroponic system'. *Iranian Journal of Plant Physiology* 9 (1), 2591-2599.

Introduction

One of the bioenvironmental problems of today's world is water and soil pollution with heavy metals, which in addition to their effect on agricultural yields endanger human health (Kabata-Pendias, 2001). Increase in the levels of heavy metals in human body can be due to consumption of plants grown in areas contaminated with these metals or irrigation with waters contaminated with them (Alexander et al., 2006). Long-term application of wastewater which is generally used in vegetable cultivation results in the concentration of heavy metals in soil and their transfer to plants at a high and dangerous level (Yargholi et al., 2007).

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Received: May, 2018
Accepted: September, 2018

Cadmium enters the air by mining, fossil fuels, and industrial wastes. Besides, phosphorus fertilizers contain high levels of cadmium, which leads to the accumulation of high amounts of this heavy metal in soils (Lefever et al., 2009).

Cadmium alters morphological, physiological, biochemical, and structural processes in plants (Mishra et al., 2006). It usually affects activities like absorption and transportation of elements such as calcium, phosphorus, potassium, and water in plants, and also the main plant processes such as photosynthesis, cell proliferation, and water absorption by plant roots (Das et al., 2000). Toxic effects of cadmium on morphological processes causes inhibition and abnormalities in general growth in many plant species (Rascio and Navari-Izzo, 2011).

Cadmium is a cause of most cancers and contributes to cardiovascular diseases and hypertension (Turkdogan, 2003; Waalkes, 2003). On the other hand, vegetables are part of human food chain and contain essential and harmful elements at a wide range of concentrations and thus contaminated vegetables are considered a threat to human health (Yanqum, 2004). Consumption of vegetables contaminated with cadmium by humans and animals results in diseases such as inflammation of the lung, inflammation of the bronchus, cancer, and hypertension (Bernard, 2008).

It is not known for certain what form of cadmium is absorbable by plants, but it seems that free metal ions are mainly absorbed from soil solutions by roots. The amount of cadmium that moves from roots to stems, leaves, and aerial organs depends on various factors such as the plant type, the soil type, and the initial cadmium concentration existing in the soil (Taylor et al., 2001).

Some reports have shown that most metals tend to remain in plant roots (Pruvot and Douay, 2006). Generally, heavy metal concentrations are higher in leafy plants than other food plants (Afyuni et al., 2003).

A study on three plants of tomato, cress, and lettuce showed that the average cadmium absorptions by all these plants were significantly different from the control and the highest and lowest absorption levels were recorded for tomato and lettuce, respectively (Abedi-Kupai et al., 2015). There are various methods to examine the effect of cadmium on plant growth and also cadmium absorption level, one of these being the aeroponic method which is employed to study cadmium effects especially at different growth stages of the plant.

The aeroponic method is one of the latest methods for plant cultivation without using soil. In this system the nutrient solution is sprayed on the roots from below. Aeroponics is a process of plant growth in an environment of mist, without the use of soil or any other media (Hayden et al., 2004). In an aeroponic system, the nutrient solution is delivered directly to the plant's root and therefore, there is no intermediate environment interrupting the delivery of nutrient solutions or contaminating it with bacteria (Hayden, 2005). Aeroponics allows easy access to the roots and thoroughly controls the area around the root, including the temperature, the nutrition levels, pH, the number and the duration of inhibitions, and the availability of oxygen (Rostami and Movahedi, 2016).

Taking into account the advantages of aeroponic system for examination of heavy metal absorption by plants and especially vegetables, the aim of this study was to examine the effects of cadmium on morpho-physiological traits of garden cress and radish in an aeroponic system

Materials and Methods

Plant selection

Taking into account the high consumption of the two plants of cress and radish in human food chain, these two plants were investigated in this study. Cress or garden cress (*Lepidum sativum*) is an annual plant, the height of which might reach 30 to 50 centimeters and its young leaf and stem have a pungent and desirable taste in fresh form (Smical et al., 2008). Wild radish (*Raphanus sativus*) is a species of the Brassicaceae family. The leaves and roots of radishes contain a large quantity of vitamin C. The radish has anticancer properties and it has been used for treatment of liver disorders for a long time (Hara et al., 2009). Seeds of radish (*Raphanus sativus*) and cress or garden cress (*Lepidum sativum*) were provided from Pakan Company.

The aeroponic system used in this study consisted of the seed container, nozzles, nutrient solution container, nutrient solution, spray jets, nutrient solution recovery pumps, and the control system.

In order to examine the cadmium effect on the growth of radish and cress plants in the aeroponic system, 3 levels of cadmium chloride (0, 3, 6 mgl^{-1}) were provided. The Hoagland nutrient solution was used for plant nutrition. Adding cadmium chloride to the nutrient solution started at three or four leaf stage.

After 40 days, the plants were taken from the aeroponic system with the whole root and the shoots and the roots were separated and the root length, the total length and the number of leaves were measured. Then, the root fresh weight and the shoot fresh weight were measured. After that, in order to assess the dry weights, they were placed in an oven with a temperature set at 72 degrees centigrade for 48 hours and the dry weights of the root and the shoot were measured. Then, the amounts of the chlorophylls a and b and carotenoid were assessed (Zhongfu et al., 2009).

In order to measure the cadmium absorption by the stems and dried roots, they were thoroughly ground to a soft powder. 0.5 grams of each stem sample and 0.2 grams of each root sample were taken for cadmium assessment and the cadmium quantity of each sample was measured separately by an atomic absorption spectroscopy (AAS) instrument. The measurement of proline was performed by using Batis et al (1973) method. For this, 0.5 grams of the sample was ground using liquid nitrogen in a porcelain mortar. Then, 10 ml of sulfosalicylic acid (3%) was added to the sample and it was ground again. The resulting solution was placed in a 10000 revolutions per minute centrifuge for 15 minutes. Two ml of the liquid was taken from the surface of the centrifuged solution and 2 ml acetic acid and 2 ml ninhydrin reagent (1.25 grams ninhydrin + 30 ml of glacial acetic acid + 20 ml of 6 M phosphoric acid) were added to it before it was mixed for 15 minutes at 40 degrees centigrade by a mixer. The obtained solution was placed in a bain-marie at 100 degrees centigrade for 1 hour. Then, it was placed in a cold water bath for 10 minutes and then, 4 ml toluene was added to it. Tube contents were intensely mixed to obtain two phases. In order to read the absorption amount in the spectrophotometry device, toluene was used as the control and in order to measure the proline amount, the absorption amount of the top phase was measured at 520 nanometer. In order to measure the protein concentrations, one gram of plant tissue and one milliliter of 50 mM tris-hcl extraction buffer with a pH of 7 containing 10 mM $MgSO_4$, 2 mM EDTA, 20 mM DTT, 10% glycerol (V/V), and 2% PVP (V/W) were ground in the mortar and then, the solution was transferred to the centrifuge tube and centrifuged at 4 degrees centigrade for 20 minutes in 13000 g by a refrigerated centrifuge machine. Then, the floating solution was collected and once again centrifuged for 20 minutes in 13000 g before it was transferred to an eppendorf. The volume of the resulting supernatant was recorded and it was placed for short term preservation at 70 degrees centigrade.

The resulting protein extracts were used to measure the protein. In order to measure the protein content in the extracts, Bradford (1976) was used. Anthocyanin content was determined in 0.3% HCl in methanol at 25° C using the extinction coefficient (33000 cm 2 mol^{-1}) at 550 nm (Wagner, 1979). For estimation of total phenol content, 0.05g plant material was extracted with boiling 80% methanol for 3 h (Conde et al., 1995). Total phenol content was determined by using Folin-Ciocalteu reagent based on Akkol et al. (2008). One ml of the methanol extract was mixed with 5 ml Folin-Ciocalteu reagent and 4 ml sodium carbonate solution 7.0 %. The mixture was kept for 2 h before its absorbance was measured at 765 nm. Gallic acid was used as a standard for the calibration curve.

The experiments were conducted as a completely randomized design with 5 replications (each replication consisting of one plantlet). The SPSS software and the Duncan's test were used, respectively for the data of this experiment and the mean comparison.

Table 1
Mean comparison for the effect of different concentrations of $CdCl_2$ on morpho-physiological characteristics of radish in an aeroponic system

Treatment	dry weight of shoot (g)	fresh weight of shoot (g)	dry weight of root (g)	fresh weight of root (g)	root length (cm)	plant height (cm)
Control	2.04±0.27 a	27.1±3.68 a	0.41±0.026 a	3.9±0.27 a	26.7±1.78 a	50.1±2.23 a
mg l^{-1} 3	1.7±0.23 ab	17.5±1.89 b	0.23±0.022 b	2.1±0.19 b	13.7±1.71 b	36.2±1.42 b
mg l^{-1} 6	0.85±0.12 b	5.9±1.67 c	0.22±0.034 b	1.9±0.25 b	13.0±1.52 b	27.3±1.35 c

Treatment	Cd in shoot (mg/kg)	Cd in root (mg/kg)	Leaf number	Total chlorophyll (mg/g)	Carotenoid (mg/g)	Chlorophyll b (mg/g)	Chlorophyll a (mg/g)
Control	00±00 c	00±00 c	18.8±2.48 a	3.3±0.21 a	0.46±0.036 a	1.1±0.099 a	1.6±0.096 a
mg l^{-1} 3	0.0037±0.0022 b	0.725±0.029 b	16.5±1.91 b	2.8±0.099 b	0.34±0.015 ab	0.8±0.032 b	1.4±0.049 b
mg l^{-1} 6	0.28±0.019 a	3.3±0.24 a	10.2±0.25 c	2.4±0.14 b	0.28±0.017 b	0.7±0.053 b	1.2±0.066 b

Treatment	total protein (mg/g FW)	proline (μ mol/g FW)	total phenol content (μg/g FW)	anthocyanin content (μM/g FW)
Control	0.3639±0.009 a	0.1943±0.0054 b	0.0441±0.0011 c	0.1435±0.81 c
3 mg l^{-1}	0.3365±0.0183 ab	0.2778±0.0068 ab	0.0562±0.0025 b	0.1599±0.062 b
6 mg l^{-1}	0.3228±0.0228 b	0.3505±0.00396 a	0.0955±0.0034 a	0.1745±0.053 a

Means followed by the same letter(s) are not significantly different at 0.05 level of probability.

Results

Number of Leaves

The effect of cadmium on the number of leaves produced in both radish and cress plants showed that there was a significant difference between cadmium chloride application and the control treatment and the highest number of the leaves in both radish and cress plants were obtained in the control 18.8 and 30.8, respectively and the least number of the leaves belonged to the 6 mgl^{-1} cadmium chloride treatment 20.7 and 10.2 for cress and radish, respectively (Tables 1 and 2).

Plant height

Results of different concentrations of cadmium chloride on the heights of the radish and cress plants grown with cadmium chloride nutrition showed that the plant heights had a significant decrease compared to the control and the highest heights were observed in the control 32.67 and 50.1 cm for cress and radish, respectively. Also, the lowest heights were recorded under 6 mgl^{-1} cadmium chloride treatment, 21.1 and 27.3 cm for cress and radish, respectively (Tables 1 and 2).

Root length

Application of cadmium chloride significantly decreased the root lengths of radish and cress. The lowest root lengths (6.5 and 13 cm for cress and radish, respectively) were obtained under 6 mgl^{-1} concentration of cadmium chloride and the highest root lengths (14.5 and 26.7 cm for cress and radish, respectively) were observed in the control treatment (Tables 1 and 2).

Root fresh and dry weights

Results of the mean comparison for the effects of different concentrations of cadmium chloride on the fresh and dry weights of the root are presented, in the Tables 1 and 2, respectively. Based on these Tables, the highest root fresh weights were recorded (2.3 and 3.9 in cress and radish, respectively) in the control while the lowest fresh weights (0. 9 and 1.9 g in cress and radish, respectively) were observed under 6 mgl^{-1}

Table 2
Mean comparison for the effect of different concentrations of $CdCl_2$ on morpho-physiological characteristics of garden cress in an aeroponic system

Treatment	dry weight of shoot (g)	fresh weight of shoot (g)	dry weight of root (g)	fresh weight of root (g)	(cm) root length	plant height (cm)
Control	1.5±0.25 a	11.3±0.95 a	0.4±0.044 a	2.3±0.26 a	14.5±1.41 a	32.6±2.38 a
mg l^{-1} 3	0.6±0.086 b	7.6±0.82 b	0.2±0.027 b	1.5±0.058 b	10.7±0.71 b	31.2±0.54 a
mg l^{-1} 6	0.5±0.067 b	4.1±0.67 c	0.09±0.21 c	0.9±0.16 c	6.5±1.17 c	21.1±2.12 b

Treatment	Cd in shoot (mg/kg)	Cd in root (mg/kg)	leaf number	total chlorophyll (mg/g)	carotenoid (mg/g)	chlorophyll b (mg/g)	chlorophyll a (mg/g)
Control	00 c	00 c	30.8±3.6 a	3.6±0.19 a	0.6±0.036 a	1.5±0.11 a	1.7±0.11 a
mg l^{-1} 3	0.02±0.009 b	0.4±0.019 b	28.1±2.99 b	3.1±0.11 a	0.4±0.015 b	0.9±0.06 b	1.5±0.05 a
mg l^{-1} 6	0.11±0.04 a	2.9±0.84 a	20.7±2.15 c	1.8±0.25 b	0.2±0.017 c	0.7±0.08 b	0.7±0.061 b

Treatment	total protein (mg/g FW)	Proline (µ mol/g FW)	total phenol content (µg/g FW)	Athocyanin content (µmol/g FW)
Control	0.3147±0.01487 a	0.1379±0.0085 b	0.0333±0.0014 c	0.1128±0.018 b
mg l^{-1} 3	0.2952±0.0119 a	0.2623±0.0068 a	0.0640±0.0019 b	0.1147±0.015 b
mg l^{-1} 6	0.2283±0.0135 b	0.2747±0.0063 a	0.0790±0.0038 a	0.1428±0.011 a

Means followed by the same letter(s) are not significantly different at 0.05 level of probability.

concentration of cadmium chloride. Control plants had the maximum root dry weights (0.34 and 0.41 g, respectively, in cress and radish) and the 6 mgl^{-1} concentration of cadmium chloride resulted in the lowest level (0.09 and 0.22 g, respectively, in cress and radish).

Fresh and dry weights of the shoot

The mean comparison (Tables 1 and 2) of the cadmium chloride effect on the fresh and dry weights of the shoot showed the lowest fresh and dry weights of the shoot in radish (5.9 and 0.85 g, respectively) and cress (4.1 and 0.5 g, respectively) under application of the 6 mgl^{-1} concentration of cadmium chloride.

Photosynthetic pigments

Results of mean comparison showed that for cress, the highest amounts of chlorophyll a (1. 7 mg/g), chlorophyll b (1.5 mg/g), and carotenoid (0.6 mg/g) were obtained under the control treatment and the lowest amount was recorded under the 6 mgl^{-1} concentration of cadmium chloride. Similar results were obtained for the radish plant and the highest amounts of chlorophyll a (1.6 mg/g), chlorophyll b (1.1 mg/g), and carotenoid (0.46 mg/g) belonged to the control while the lowest was obtained under 6 mgl^{-1} concentration of cadmium chloride.

Total protein

Under Cd stress, a significant increase was observed in total protein in garden cress and radish compared to the control**.** The mean comparison (Tables 1 and 2) of the cadmium chloride effect on the total protein showed the highest total protein contents of the radish and cress were recorded under control treatment 0.3639 mg/g FW and 0.3147 mg/g FW were, respectively.

Proline

Cd stress caused increase in proline in both plants studied. Results showed that the highest proline (0.2747 and 0.3505 µ mol/g FW, respectively, in cress and radish) were obtained under 6 mgl^{-1} concentration of cadmium chloride (Tables 1 and 2).

Total phenol content

Total phenol content increased at high concentrations of $CdCl_2$ in garden cress and radish**.**

Results of mean comparison (Tables 1 and 2) showed that the highest amounts of total phenol content were obtained under 6 mgl^{-1} concentration of cadmium chloride in garden cress and radish, 0.0790 and 0.0955 µg/g FW, respectively.

Anthocyanin content

Anthocyanin content showed a significant increase in both plant (garden cress and radish) after exposure to $CdCl_2$. Based on the results of mean comparison, the highest anthocyanin contents (0.1428 and 0.1745 µM/g FW, respectively, in cress and radish) were obtained under 6 mgl^{-1} concentration of cadmium chloride (Tables 1 and 2). .

Cadmium content

Results showed that with an increase in the amount of cadmium, its accumulation in the root and the shoot of cress and radish increased, so that under 3 mgl^{-1} concentration of cadmium chloride, the cadmium content of the radish root was 0.725 mg/kg and this reached 3.3 mg/kg under 6 mgl^{-1} concentration of cadmium chloride. The amounts of cadmium in the shoot of this plant has a similar pattern (0.0037 and 0.28 mg/kg in, respectively, 3 and 6 mgl^{-1}). Besides, under 3 mgl^{-1} concentration of cadmium chloride, the cadmium content was 0.4 mg/kg in the cress root and 0.02 mg/kg in the cress shoot while under 6 mgl^{-1} concentration of cadmium chloride, the cadmium amount was 2.9 mg/kg in the cress root and 0.11 mg/kg in the shoot.

Discussion

Cadmium is one of the most important heavy metals found in soils, which limits plant growth all around the world. This metal tends to accumulate in plant tissues and has negative effects on plant performance and growth.

In this experiment, the cadmium effects on the most characteristics under study for both cress and radish plants were negative. Decrease in the number of leaves associated with increase in cadmium concentrations has been reported in some plants, such as red beet (Behtash et al., 2010) and *Phaseolus mungo* (Ali-Khan, 2012). A study on the wheat indicated that with increase in cadmium concentrations of the soil, the length of the shoot decreased (SohrabiYourtchi and Bayat, 2013). This height reduction in high concentrations can be due to its hindering the cell division and meristem activity (Davies et al., 2002). Another study also showed that with an increase in the cadmium concentration, the root length decreased for the *Brassica juncea* plant (John et al., 2009). This reduction in the root length can be due to the suppression of the cell length growth as a result of irreversible inhibition of protein pumps by cadmium (Aididi and Okamote, 1993). Yadegari (2018) who studied plants exposed to heavy metal found that different levels of nickel and cadmium had significant effects on the morphological and physiological characteristics of purslane and increased heavy metals concentration decreased significantly these characteristics.

Cytokinins are growth hormones, which cause cell proliferation and, consequently, plant growth. Based on the research conducted, it has been reported that one of the factors capable of weight reduction in plant organs is reduction in the activities of cytokinin hormone in the presence of cadmium (Mok, 1994). It has been reported that with increase in the cadmium concentrations in cress and radish plants, the fresh and dry weights have decreased (Khorsandi et al., 2016). Decrease in the fresh and dry weights of both roots and shoots under cadmium treatment have been reported compared to the control for plants such as lettuce (Dias et al., 2013), beans (Bahmani et al., 2012), barley (Sadlou et al., 2014), and safflower (Morady and Ehsanzadeh, 2014), which is in accordance with the results obtained in this study. Under high cadmium concentrations, this element affects some cellular parameters (photosynthesis, nutrient accumulation, oxidative stress and cellular toxicity), which affects biomass accumulation and thus, weight reduction (Azevedo et al., 2005; Monteiro et al., 2012).

In many plants such as wheat (Moussa and EL-Gamal, 2010), cress (Gill et al., 2012), and tomato (Ammar et al., 2008), the amounts of photosynthetic pigments decreases with cadmium treatment. Many studies show that the initial effect of cadmium is on the activities of photosynthetic pigments, especially the production of chlorophyll and carotenoid (Baszynski et al., 1980). Baryla et al. (2001) showed that in some instances the effect of cadmium is through reduction of chloroplast density. It is possible that cadmium directly participates in cell proliferation and division and stomatal conductance by cadmium intensely decreases. In many plant species, the structure and performance of the chloroplast is affected by cadmium (Atal et al., 1991).

In this study, the amounts of cadmium accumulation in both cress and radish plants were more in the roots than in shoots. Cadmium is absorbed by the root and transmitted to the shoot. These results are in accordance with the results by Khorsandi and colleagues (2016).

According to result of the present experiments, protein content decreased under Cd stress condition and similar results were obtained by Farzami Sepehr (2015) in *Brassica oleracea* L.

This study has examined cadmium effect on cress and radish performance in an aeroponic system for the first time. The aeroponic method can be used in studies related to mineral nutrition, study of water efficiency, study of nitrification, plant disease studies, examination of materials released by plants, examination of temperature effect, selection of mutant roots, plant organ and tissue culture, and the study of gravitation effects on plant growth. The system is suggested in the studies of the effects of nutritive elements on root growth where the direct effects of elements on roots can be monitored in a controlled environment.

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