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ORIGINAL ARTICLE

Protective Effect of Humic acid and Chitosan on Radish (*Raphanus sativus*, L. var. sativus) Plants Subjected to Cadmium Stress

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Background

Humic acid or chitosan has been shown to increase plant growth, yield and improving physiological processes in plant, but its roles on alleviating the harmful effect of cadmium on plant growth and some physiological processes in plants is very rare. Pot experiments were conducted to study the role of 100 and 200 mg/kg dry soil from either humic acid or chitosan on counteracted the harmful effects of cadmium levels (100 and 150 mg/kg dry soil) on radish plant growth and some physiological characters

Results

Cadmium at 100 and 150 mg kg^{-1} soil decreased significantly length, fresh and dry weights of shoot and root systems as well as leaf number per plant in both seasons. Chlorophyll, total sugars, nitrogen, phosphorus, potassium, relative water content, water deficit percentage and soluble proteins as well as total amino acids contents were also decreased. Meanwhile, cadmium concentration in plants was increased. On the other hand, application of chitosan or humic acid as soil addition at the concentration of 100 or 200 mg kg^{-1} increased all the above mentioned parameters and decreased cadmium concentrations in plant tissues. Chitosan at 200 mg kg^{-1} was the most effective than humic acid at both concentrations in counteracting the harmful effect of cadmium stress on radish plant growth.

Conclusion

In conclusion, both natural chelators, in particular, chitosan at 200 mg/kg dry soil can increase the capacity of radish plant to survive under cadmium stress due to chelating the Cd in the soil, and then reduced Cd bio-availability.

Key words: Humic, Chitosan, Cadmium, Radish, Growth

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Heavy metals make a significant contribution to environmental pollution as a result of human activities such as mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping and military operations (Nedel-Koska and Doran, 2000). They present a risk for primary and secondary consumers and ultimately humans. Due to high Cd^{2+} mobility in the soil-plant system it can easily enter food chain and create risk for humans, animals, plants and the whole environmental of our modern society (Pinto et al. 2004). A part of agricultural soils all over the world is slightly to moderately contaminated by cadmium (Cd) due to extended use of super-phosphate fertilizers, sewage sludge application as well as smelters dust spreading and atmospheric sedimentation (Thawornchaisit and Polprasert, 2009). According to Wagner (1993), Cd concentration in soil solution of uncontaminated soils is in the range of 0.04-0.32 µM, while moderately polluted soils contain 0.32-1.00 µM. In soil, containing more than 35 µM Cd in the soil solution, only species with Cd tolerance are capable of surviving. Cadmium has been shown to cause many morphological, physiological and biochemical changes in plants, such as growth inhibition, and water imbalance (Benavides et al., 2005). Cadmium produces alterations in the functionality of membranes, decreases chlorophyll content, and disturbs the uptake and distribution of macro- and micro-nutrients in plants (Ramon et al., 2003).

Several strategies have been proposed for applicable and cost effective methods for polluted soils remediation. Phytoremediation, the use of hyperacumulator plants as *Thlaspi caerulescens* to extract heavy metals from soils and groundwater, has revealed great potential. However, it is limited by the fact that plants need time, nutrient supply and, the limited metal uptake capacity. The second approach recommends profitable use of synthetic chelators such as EDTA which have shown positive effects in enhancing heavy metal extraction through phytoremediation, but they have also revealed a vast number of negative side-effects on the soil. It is a non-selective agent which could extract various cations, including alkaline earth cations, such as calcium and magnesium, which are necessary for plant growth (Barona et al., 2001).

As an alternative to these synthetic chelators, widespread natural sources, such as humic substances and chitosan could be used. Humic acid contain acidic groups such as carboxyl and phenolic OH functional groups. Therefore, it provides organic macromolecules with an important role in the transport, bioavailability and solubility of heavy metals (Chen and Zhu, 2006). The multiple effects which humic substances or chitosan exert on plant growth can be grouped into indirect effects on physiological, chemical and biological properties of the soil, and direct effects on physiological processes of plant (Ohta et al., 2004). Chitosan, natural polysaccharides, is an essential chemical component in the development of plants owing to its excellent behavior in biodegradability, and non-toxicity. Little is known about the effects of chitosan and humic acid on alleviating the harmful effect of cadmium on plant growth and some physiological characters. Therefore, the objective of this investigation is to investigate and clarify the toxic effects of cadmium on growth, ion content as well as certain physiological aspects of radish plants. Moreover, using humic acid or chitosan as an alternative to synthetic chelators were studied to assess if their application could provide useful recovery the adverse effects of cadmium toxicity. Another objective is to assess the alleviative effects of humic acid and chitosan, as alternatives to synthetic chelators on Cd-impacted radish plants.

MATERIALS AND METHODS

Two pot experiments were carried out during the two successive seasons (2007 and 2008) in the greenhouse of Plant Pathology Department, Mansoura University. Closed plastic pots (30 cm in diameter) were filled with 8 kg soil. Then the pots were divided into three sets and contaminated with cadmium chloride at concentrations of 0, 100 and 150 mg kg-1 dry soil (Table, 1), Cd doses were added dissolving in the first irrigation water. In each set the pots were divided into 5 groups and treated with either humic acid or chitosan, both at 100 and 200 mg kg^{-1} soil or left untreated as a control. Twenty uniform seeds of radish (*Raphanus sativus*, L. var. sativus) were sown on 10th April in the previous pots and irrigated with tap water when required. The pots were arranged in a complete randomized block design with three replications. Three weeks after sowing, plants were thinned to leave 5 uniform young plants per pot. At harvest (45 days from sowing) , length of both the root and shoot systems and their fresh and dry weights as well as leaf number per plant were recorded in addition to the following stress-indicative biochemical parameters.

Chlorophylls content was extracted by methanol for 24 hour at laboratory temperature after adding traces from sodium carbonate, and determined spectrophotometrically. Chlorophylls content in leaves was calculated according to Lichtenthaler and Wellburn (1985). Nutrient concentrations in shoots were determined including total nitrogen by microkjeldahl method and potassium using flame photometer (Kalra, 1998), and phosphorous using ammonium molybdate and ascorbic acid (Cooper, 1977). Cadmium concentration in plants was determined after digesting 0.2 g from the plant dry sample by 5 cm3 from the mixture of sulphuric acid, perchloric acid and nitric acid as described by Chapman and Pratt (1982) using atomic absorption spectrophotometer. Total sugars content in the shoots was estimated using the anthrone method as described by Sadasivam and Manickam (1996). Soluble proteins concentration was measured using bovine serum albumin as standard at 595 nm according to the method of Bradford (1976). Water content was determined according to Fernandez-Ballester et al. (1998). Meanwhile the relative water content (RWC) was determined following the method of Sanchez et al. (2004), where leaves were weighted to obtain fresh weight (FW) then floated in distilled water to determine the turgid weight (TW), and then the plant materials were placed in a pre-heated oven at 80 ºC for determined dry weight (DW). RWC (%) = {(FW-DW)/ (TW-DW)} x100. The water deficit is calculated by the formula presented by Tourneux et al. (2003).

Statistical analysis: The data were analyzed following Analysis of Variance (ANOVA) and mean separations were adjusted by the Multiple Comparison test (Norman and Streiner, 2003) using the statistical computer programme MSTAT-C v.1.2

RESULTS

Growth parameter:

Generally, severe reduction in plant growth, manifested by smaller, chlorotic, wilted, and rolled leaves was recorded due to cadmium toxicity. Results in Table (2) and Figure (1) show that contaminated soil with cadmium at both concentrations decreased significantly plant growth characters (length of both shoot and root systems, their fresh and dry weights and as well as root thickness and leaf number per plant) in both seasons. High cadmium concentration had more deleterious effects on plant growth than low concentration. The reduction in root growth was more pronounced by Cd-stress than shoot.

Addition of humic acid or chitosan notably 200 mg kg^{-1} soil chitosan improved plant growth through increasing all the above mentioned growth parameters in polluted or nonpolluted soils under such cadmium levels. Chitosan at 200 mg kg^{-1} soil was the superior treatment in in alleviating the harmful effects of high cadmium concentration on radish plant.

Chlorophyll and total sugar contents:

Chlorophylls $_{a\ and\ b}$ and total chlorophylls as well as total sugar concentrations in radish leaves under the effect of both chelators and cadmium are given in Table (3). It is evident that the presence of cadmium in the soil had a negative effect on chlorophyll and total sugars contents. Their contents decreased significantly with increasing cadmium concentrations, compared to control plants. Moreover, the reduction in Chl $_b$ was extremely sharp which resulted in higher $Chl_{a:b}$ ratio as the concentration of Cd increased in the soil (Table, 3). The presence of chelators especially 200 mg/kg soil chitosan counteracted the adverse effect of cadmium on chlorophyll and total sugar contents which increased under such cadmium concentration (Figure, 2). Moreover, chelators mixed with soil increased significantly chl $_b$ which resulted in a decrease in the $chl_{a:b}$ ratio (Table, 3).

Water status

Results in Table (4) indicate that increasing concentration of cadmium up to 150 mg/kg soil decreased significantly relative water content and water content of radish plant as compared to control. On the other hand, addition of chelators to soil increased significantly water content and relative water content but decreased water deficit percentage.

The addition of both chelators specially chitosan at 200 mg/kg alleviated the harmful effect of cadmium on water status in radish leaves where application of chelators specially 200 mg/kg soil increased significantly RWC and Water content but decreased water deficit percentage as compared to untreated cadmium impacted plants (Figure 3).

Soluble proteins and total free amino acids:

Cadmium level up to 150 mg kg^{-1} soil significantly reduced the total free amino acids and soluble protein content in the shoots of radish plants. The highest reduction was observed under high cadmium concentration (Table, 5). On the other hand application of soil chelators increased significantly both soluble proteins and total free amino acids. Chitosan at 200 mg kg^{-1} soil was the most effective in this concern. Data in the same table clearly indicate that application of chelators under all cadmium levels increased significantly the content of total free amino acids and total soluble proteins as compared with untreated plants under such cadmium level (Figure 4).

Bioaccumulation of cadmium and nutrient content:

The data in Table (6a) show that increasing cadmium concentration in the soil up to150 mg kg^{-1} soil increased significantly the concentration of cadmium in plant tissue whereas decreased significantly the concentration of nitrogen, phosphorous and potassium. On the other hand, application of humic acid or chitosan as soil addition decreased significantly cadmium concentration in radish plant growing in the presence of cadmium as well as increased the content of nitrogen, phosphorous and potassium as compared to untreated plants.

Data in the same illustrated in Figure (5) indicate that addition of chelators to the soil stimulate plant growth under low and high cadmium concentration due to its role in increasing beneficial nutrient uptake as potassium, phosphorous and nitrogen as well as decreasing significantly the cadmium uptake as compared to untreated plant under such cadmium levels. Chitosan at 200 mg/kg soil was the most effective in this concern.

Table 1. Physiochemical analysis of soil used in three experiments

Soil properties		Values
Particle size distribution (%)	Sand	19
	Silt	29
	Clay	52
	Soil texture	Clay
Bulk density (g cm^{-3})		1.24
Field capacity (%)		33
EC (dSm^{-1})		1.43
pH (Soil paste)		7.6
Calcium carbonate (%)		3.7
Organic matter (%)		1.65
Soluble cations (meq L^{-1})	Ca^{2+}	5.36
	Mg^{2+}	3.23
	Na^{+}	5.28
	K^{+}	0.28
Soluble anions (meq L^{-1})	CO_3^{2-}	0
	HCO_3^{-}	4.21
	Cl^{-}	6.74
	SO_4^{-}	3.20
Available nutrients (mg Kg^{-1})	Nitrogen	43
	Phosphorus	14
	Potassium	289
Available cadmium (mg Kg^{-1})		1.50

Table 2a. Shoot fresh weight (g), Shoot dry weight (g), leaf number per plant, shoot length (cm), tap root length (cm) and tap root thickness (cm) of radish plants as affected by cadmium levels in the two growing seasons

Cadmium chloride (mg/g Soil DW)	Shoot fresh weight		Shoot Dry weight		Leaf number/ plants		Shoot length (cm)		Tap Root length (cm)		Tap root thickness (cm)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
0	40.52	39.62	3.57	3.47	6.86	6.53	24.9	24.89	12.7	12.46	1.74	1.74
100	30.27	29.40	2.73	2.60	6.13	5.80	22.4	22.01	10.7	10.65	1.42	1.42
150	32.22	23.92	1.93	1.92	5.26	4.93	20.2	19.76	9.80	9.85	1.20	1.20
LSD at 0.05	1.35	0.846	0.126	0.067	0.415	0.332	0.679	0.210	0.513	0.320	0.058	0.059

Table 2b. Shoot fresh weight (g), Shoot dry weight (g), leaf number per plant, shoot length (cm), tap root length (cm) and tap root thickness (cm) of radish plants as affected by chelators levels in the two growing seasons

Chelators (mg/Kg soil)	Shoot fresh weight		Shoot Dry weight		Leaf number/ plants		Shoot length (cm)		Tap Root length (cm)		Tap root thickness (cm)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
0	24.33	24.08	1.84	1.74	4.66	4.33	19.3	19.31	9.47	9.53	1.08	1.08
CHI100	28.22	28.72	2.61	2.54	6.22	5.88	22.2	21.97	10.9	10.9	1.44	1.44
CHI200	38.55	36.12	3.37	3.20	6.77	6.44	24.2	23.57	11.8	11.6	1.62	1.62
HA100	31.42	32.02	2.74	2.80	6.33	6.00	23.0	22.87	11.4	11.2	1.52	1.52
HA200	34.17	33.96	3.15	3.04	6.44	6.11	23.8	23.36	11.7	11.5	1.61	1.61
LSD at 0.05	1.74	1.094	0.165	0.086	0.534	0.430	0.877	0.274	0.662	0.412	0.075	0.076

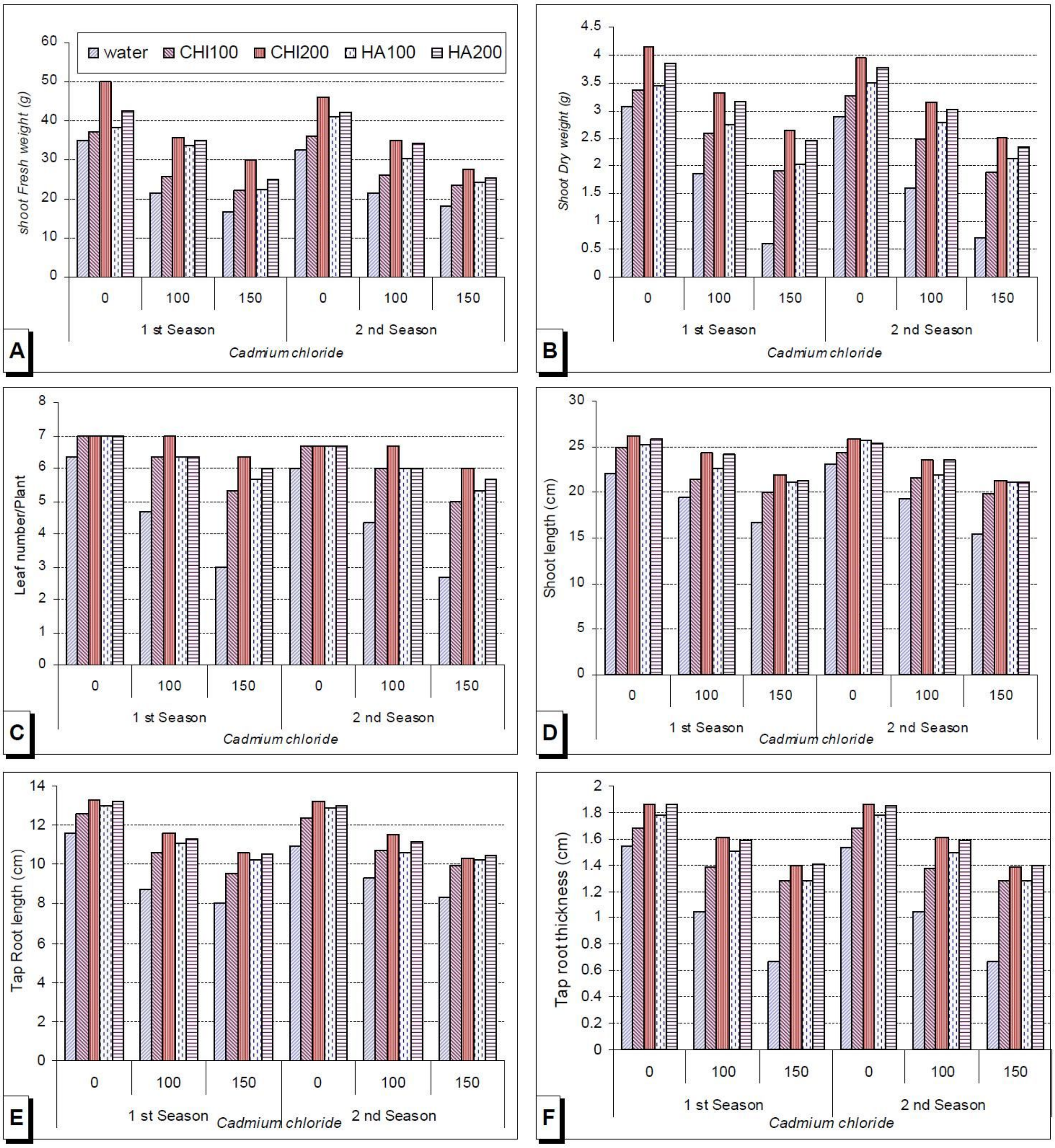

Figure 1 Shoot fresh weight (g), Shoot dry weight (g), leaf number per plant, shoot length (cm), tap root length (cm) and tap root thickness (cm) of radish plants as affected by the interaction between cadmium levels and chelators in the two growing seasons

Table 3a. Chlorophyll content (mg/g fw), chlorophyll a:b ratio and total sugars (mg/g dw) content of radish plants as affected by cadmium levels in the two growing seasons

Cadmium chloride (mg/g Soil DW)	Chlorophyll A		Chlorophyll B		Chlorophylla:b ratio		Total chlorophyll		Total sugars	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
0	1.111	1.101	0.810	0.798	1.476	1.482	1.924	1.899	2.475	2.384
100	1.066	1.057	0.474	0.551	2.465	2.123	1.590	1.608	1.875	1.761
150	0.974	0.985	0.319	0.398	3.186	2.709	1.294	1.384	1.537	1.439
LSD at 0.05	0.020	0.020	0.025	0.012	0.149	0.159	0.0193	0.0182	0.0332	0.040

Table 3b. Chlorophyll content (mg/g fw), chlorophyll $_{a:b}$ ratio and total sugars content (mg/g dw) of radish plants as affected by chelators levels in the two growing seasons

Chelators (mg/Kg soil)	Chlorophyll A		Chlorophyll B		Chlorophyll$_{a:b}$ ratio		Total chlorophyll		Total sugars	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
0	0.941	0.937	0.314	0.310	3.311	3.350	1.255	1.248	1.449	1.393
CHI100	1.034	1.030	0.479	0.542	2.526	2.040	1.515	1.573	1.868	1.772
CHI200	1.098	1.093	0.705	0.765	1.798	1.546	1.803	1.858	2.321	2.228
HA100	1.057	1.074	0.548	0.611	2.230	1.878	1.624	1.685	1.999	1.888
HA200	1.053	1.104	0.624	0.578	2.013	1.711	1.733	1.788	2.126	2.026
LSD at 0.05	0.025	0.032	0.033	0.043	0.192	0.189	0.0248	0.0241	0.0427	0.051

DISCUSSION

Cadmium is a non-essential element that negatively affects plant growth and development. It is released into the environment by power stations, heating systems, metal-working industries or urban traffic. It is recognized as an extremely significant pollutant due to its high toxicity and large solubility in water (Pinto et al., 2004). Cadmium has been shown to cause many morphological, physiological and biochemical changes in plants, such as growth inhibition, and water imbalance (Benavides et al., 2005). The reduction in both radish shoot and root system under cadmium stress may be attributed in part to the inhibition of mitosis, the reduced synthesis of cell-wall components, and changes in the polysaccharides metabolism (Punz and Sieghardt, 1993). Moreover, cadmium stress can interfere with a number of metabolic processes such as water and nutrient uptake and photosynthesis (Sheoran et al., 1990) which play a critical role in plant growth. In addition, cadmium decreased cell turgor potential and cell wall elasticity leading to formation of small cells and intercellular space areas (Barcelo et al., 1988). On contrast, application of both chelators decreased increased significantly shoot and root systems growth under polluted and unpolluted soils. The beneficial effect of humic acid (HA) and Chitosan (CHI) on plant growth may be attributed to the promoting effects on nutrients uptake and nutritional status especially nitrogen, potassium and phosphorous (Table,6) which are necessary for plant growth as well as decreasing cadmium concentration in plant organs (Table, 6). The stimulating effects of humic substances on plants growth have been reported, i.e., increase dry weight of shoot, root growth, plant height and macronutrient uptake in oat plants (Rosa et al., 2004); enhance phosphorus uptake and dry matter production in corn (Andrade et al., 2004); stimulate growth and leaf N and chlorophyll content in wild olive (Murillo et al., 2005) and increased root and leaf dry weight, root diameter, root length as well as

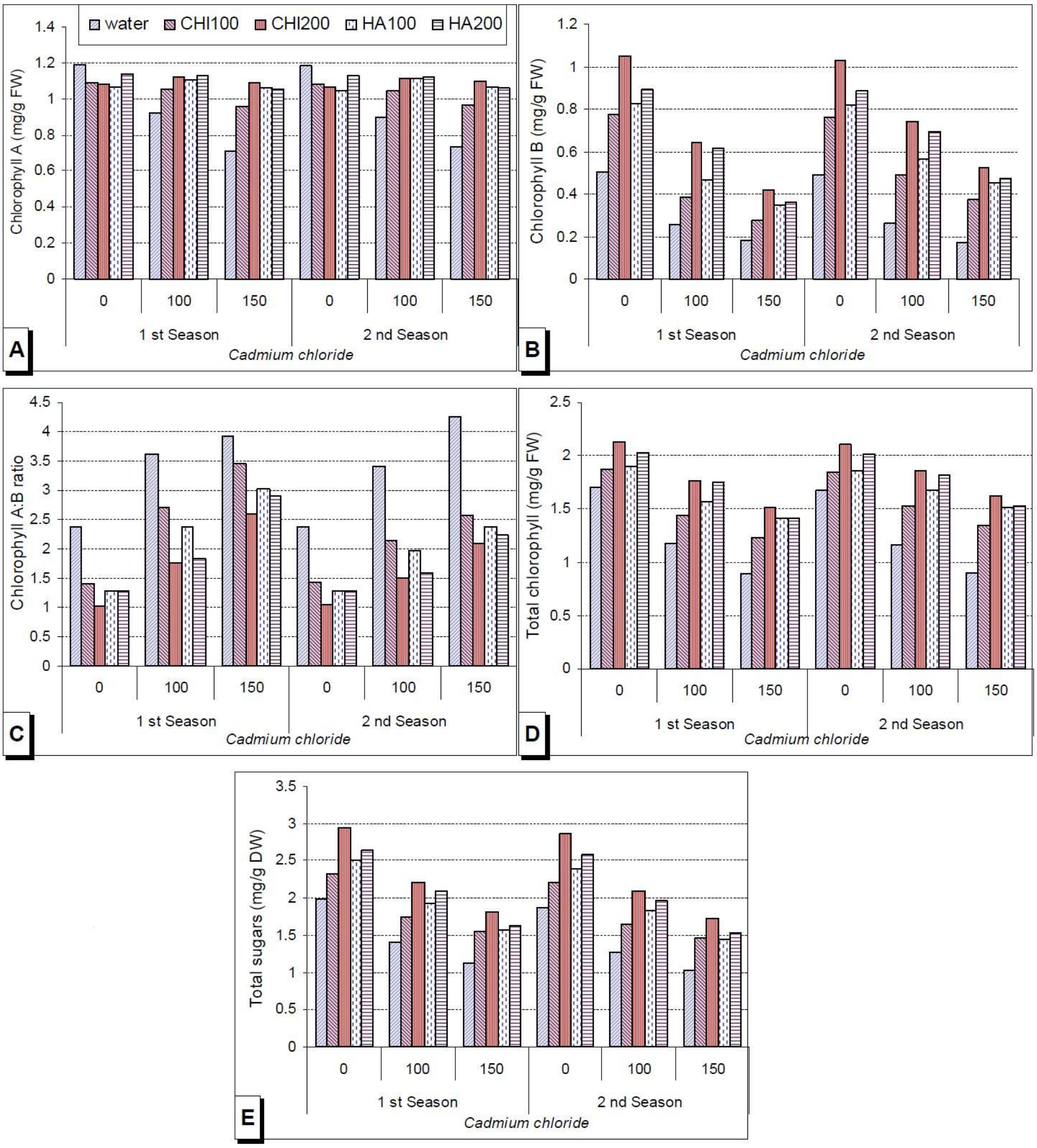

Figure 2 Chlorophyll content (mg/g FW), chlorophyll $_{a:b}$ ratio and total sugars content(mg/g DW) of radish shoots as affected by the interaction between cadmium levels and chelators in the two growing seasons

Table 4a. Water content and relative water content percentage of radish leaves as affected by cadmium levels in the two growing seasons

Cadmium chloride (mg/g Soil DW)	Water content %		Relative Water content %	
	1st season	2nd season	1st season	2nd season
0	89.92	89.91	81.06	80.60
100	88.08	87.88	71.85	75.61
150	86.04	86.06	66.12	66.36
LSD at 0.05	0.2555	0.145	0.523	1.371

Table 4b. Water content and relative water content percentage of radish leaves as affected by chelators levels in the two growing seasons

Chelators (mg/Kg soil)	Water content %		Relative Water content %	
	1st season	2nd season	1st season	2nd season
0	85.27	84.93	65.94	73.18
CHI100	87.98	88.00	71.18	70.89
CHI200	89.90	89.40	78.21	78.08
HA100	88.45	88.49	73.54	73.19
HA200	88.94	88.95	76.19	45.60
LSD at 0.05	0.3300	0.188	0.676	1.770

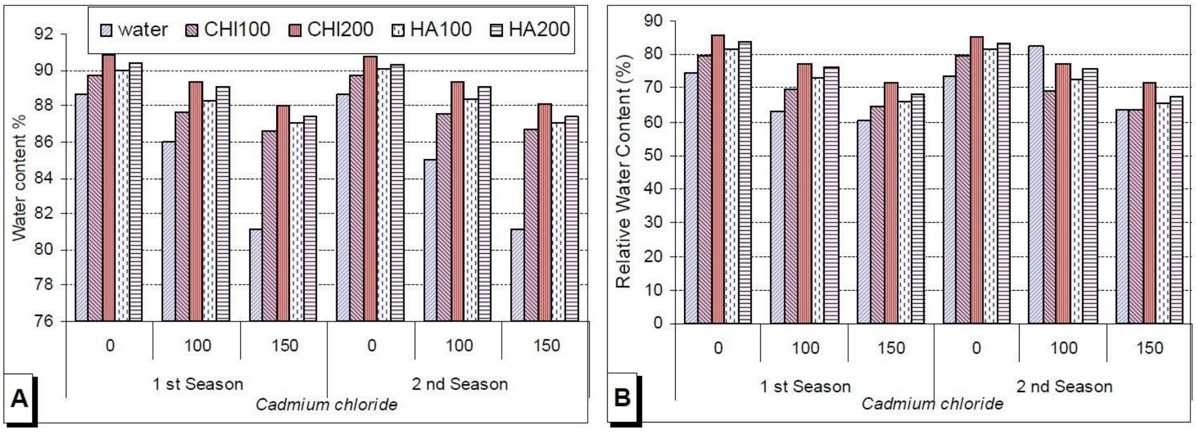

Figure 3 Water content and relative water content percentage of radish leaves as affected by the interaction between cadmium levels and chelators in the two growing seasons

Table 5a. Soluble protein (mg/g FW) and Total free amino acids (mg/g DW) content of radish leaves as affected by cadmium levels in the two growing seasons

Cadmium chloride (mg/g Soil DW)	Soluble protein		Total free amino acids	
	1st season	2nd season	1st season	2nd season
0	4.013	3.903	5.330	5.245
100	3.011	2.920	4.291	4.168
150	2.406	2.277	3.518	3.421
LSD at 0.05	0.1039	0.086	0.075	0.062

Table 5b. Soluble protein (mg/g FW) and Total free amino acids (mg/g DW) content of radish leaves as affected by cadmium levels in the two growing seasons as affected by chelators levels in the two growing seasons

Chelators (mg/Kg soil)	Soluble protein		Total free amino acids	
	1st season	2nd season	1st season	2nd season
0	2.262	2.153	3.350	3.261
CHI100	2.998	2.906	4.190	4.086
CHI200	3.767	3.658	5.094	5.00
HA100	3.233	3.113	4.472	4.361
HA200	3.456	3.336	4.792	4.711
LSD at 0.05	0.1342	0.111	0.097	0.081

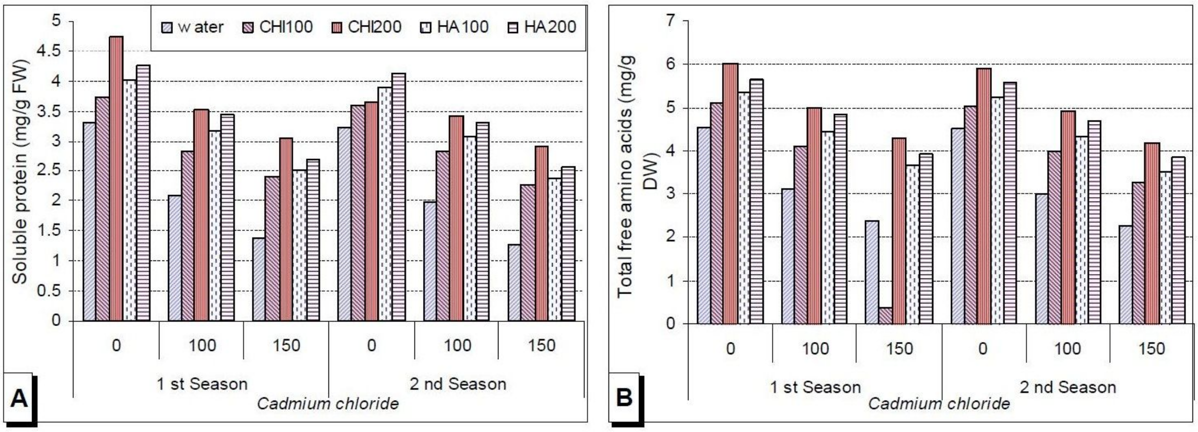

Figure 4 Soluble proteins (mg/g FW) and Total free amino acids (mg/g DW) content of radish plant as affected by the interaction between cadmium levels and chelators in the two growing seasons

Table 6a. Cadmium content and nitrogen, Phosphorous and potassium percentage of radish shoot as affected by cadmium levels in the two growing seasons

Cadmium chloride (mg/g Soil DW)	Cadmium		Nitrogen %		Phosphorus %		Potassium %	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
0	14.00	13.00	4.07	3.96	0.412	0.407	4.20	4.09
100	22.00	22.20	3.73	3.75	0.394	0.389	3.88	3.78
150	28.00	27.20	3.45	3.52	0.384	0.377	3.55	3.64
LSD at 0.05	0.433	0.511	0.074	0.028	0.0075	0.002	0.119	0.038

Table 6b. Cadmium content and nitrogen, Phosphorous and potassium percentage of radish shoot as affected by chelators levels in the two growing seasons

Chelators (mg/Kg soil)	Cadmium		Nitrogen %		Phosphorus %		Potassium %	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
0	36.66	35.88	3.52	3.46	0.38	0.35	3.52	3.48
CHI100	22.50	21.30	3.68	3.74	0.39	0.39	3.83	3.83
CHI200	13.00	12.30	3.94	3.89	0.40	0.40	4.21	4.02
HA100	20.83	20.08	3.73	3.79	0.40	0.39	3.99	3.89
HA200	15.83	15.01	3.81	3.85	0.40	0.40	3.81	3.96
LSD at 0.05	0.559	0.660	0.096	0.095	0.009	0.002	0.165	0.049

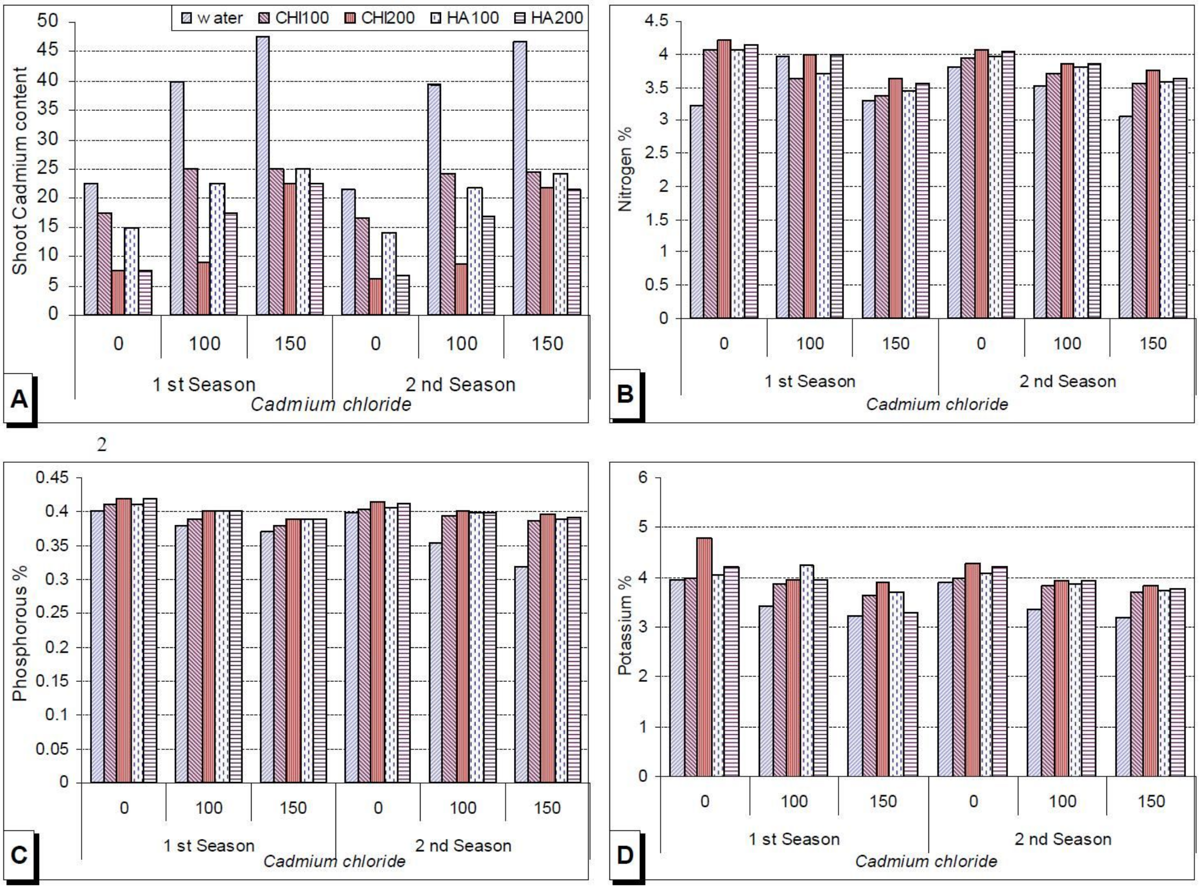

Figure 5. Cadmium content and nitrogen, Phosphorous and potassium percentage of radish shoot as affected by the interaction between cadmium levels and chelators in the two growing seasons

leaf fresh weight and leaf crude proteins in forage turnip (Albayrak and Camas, 2005). The mechanism by which humic acid stimulate plant growth are not fully clear, although there are some theories which probably work together. It is well established that HA have beneficial physical, biological and chemical effects on soils and many researchers have demonstrated that these effects can increase plant growth. Other mechanisms which have been suggested to account for promotion of plant growth by humic substances include: enhanced uptake of metallic ions and increases in cell permeability (Chen and Aviad, 1990). Moreover, the positive influences of HA on plant growth, which seem to be concentration-specific, could be mainly due to hormone-like activities of the humic acids through their involvement in cell respiration, photosynthesis, oxidative phosphorlation, protein synthesis, and various enzymatic reactions (Chen and Aviad, 1990; Muscolo et al., 1999). Chitin and chitosan are the most abundant polysaccharide compounds found in nature. They have specific properties of being environmentally friendly and easily degradable. In agriculture, CHI has been used as fertilizer and in controlled agrochemical release, to increase plant productivity (New et al., 2004, Farouk et al., 2008), and to stimulate plant growth under normal and contaminated conditions. Stimulation of plant growth was supported with the results of the current study which indicate the application of chitosan under normal or contaminated conditions stimulates radish plant growth. Moreover, Cho et al (2008) indicated that chitosan treatment increased total weight as well as length and thickness of sunflower hypocotyls compared with control. Chitosan as a carbon sources may stimulate the growth of beneficial microbes in the soil, accelerating the transferring processes of organic matter into inorganic matter and facilitating the root system of plants to absorb more nutrients from soil hence stimulating plant growth.

In the present study, cadmium exposure significantly decreased photosynthetic pigments concentrations in comparison with control plants. Reduction in photosynthetic pigments by excess cadmium has been reported by some author i.e. Abdel-Latif (2008). Decreased chlorophyll content associated with heavy metal stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis i.e. 5-aminolaevulinic acid dehydrates and protochlorophyllide reductase (Lanaras et al., 1993) or to inducing its degradation due to formation of free radicals of polyunsaturated fatty acids resulted from enhanced lipoxygenase activity. In addition, Cadmium-induced decrement in chlorophyll content was attributed to impairment in the supply of magnesium and iron to the leaves (Greger and Ogren, 1991). Alternatively, cadmium may substitute magnesium in chlorophyll molecules (Kupper et al., 1998). The reduction in Chl b was extremely sharp which resulted in higher Chl $_{a:b}$ ratio as the concentration of Cd increased in the soil (Table, 3). The increase in the ratio of Chl $_{a:b}$ have been linked with the change in pigment composition of photosynthetic apparatus which possesses lower level of light harvesting chlorophyll proteins (LHCPs) (Loggini et al., 1999). The reduction in LHCPs content is an adaptive defense mechanism of chloroplasts, leaves and plants which allows them to endure the adverse conditions (Asada et al., 1998). Reduction in total sugar content induced by cadmium treatments may be due to its inhibitory effect on photosynthetic activities, photosynthetic pigment concentrations (Table, 3) as well as on the activity of ribulose diphosphate carboxylase leading to decrease in all sugar fractions (Stibrova et al., 1986). The role of chelators on increasing chlorophylls and sugars contents under normal or polluted soils with cadmium may be attributed to increasing nutrients uptake (Table 6) specially nitrogen and potassium which increased number of chloroplast per cell as well as photosynthetic efficiency and increased sugar content in plants (Table, 3). In addition application of HA inhibit indoleacetic acid (IAA) oxidase, thereby hindering destruction of this plant growth hormone (Mato et al., 1972). Moreover, Farouk et al (2008) found that cucumber accumulated the highest carbohydrates content due to treatment with chitosan.

Heavy metals have an influence on plant-water status, causing a direct reduction in the absorption surface by inhibiting the formation of root hairs. The highest reduction in RWC was observed under high cadmium concentration. Decreased water content in different plant species growing in the presence of toxic levels of heavy metals was previously reported (Gouia et al., 2000). According to Barcelo and Poschenrieder (1990), water uptake reduction may be mediated through the following effects of heavy metals: 1) decreased root elongation, 2) decreased rate of assimilates movement from shoots to roots, 3) decreased hydraulic permeability and conductivity, 4) loss of endodermis integrity, 5) increased root suberization and lignification, and 6) increased rate of root tip dieback. On the other hand application of both chelators increased significantly root growth as root length and thickness (unpublished results) in addition to increasing the diameter of vascular cylinder and the dimension of metaxylem vessels (unpublished results) which increased the plants absorption ability. Moreover, application of chitosan assisted in conserving water in the plants by closing the stomata and decreasing transpiration (Bitteli et al., 2001) hence increasing relative water content in the leaves.

Cadmium level up to 150 mg/kg soil significantly decreased amino acid and protein content in the shoot and root of radish plants. Since the nitrogen content of the cadmium treated plants was reduced, ultimately, amino acids and protein contents of the plants were also reduced (Mayz and Cartwright, 1984). Similar results were reported by Hegazy (2001) on radish and faba bean plants. On the other hands, application of chelators counteracted the harmful effect of cadmium on total amino acid and soluble proteins due to their effect on increasing the uptake of nitrogen to the plant which is a precursor of amino acids.

The reduction in nitrogen content under cadmium treatment was comparable with the results reported by Rother et al (1983). Cd^{2+} reduced the absorption of nitrate by about 70%, due to inhibition of nitrate reductase activity in the roots (Hernandez et al, .1996). Toxicity may result from the binding of metals to sulphydryl groups in proteins, leading to inhibition of activity or disruption of structure, or from displacement of essential elements, resulting in deficiency effects (Van Assche and Clijsters, 1990). Heavy metals are able to interact with essential nutrients, thus exerting a significant influence on plant nutrient uptake. Cadmium was reported to reduce the uptake of nitrogen, phosphorus and potassium (Narwal et al., 1993). Due to its effect on plant water relationships causing a direct reduction in the absorption surface by inhibiting the formation of root hairs, and reduce membrane permeability (Barcelo and poschenriederi, 1990). A large increase of nutrient uptake was recorded for the application of HA The increased nutrient availability by HA as evident from the literature would have resulted in better absorption and higher uptake of nutrients by radish plants due to enhanced microbial activity as well as increased root growth which would have facilitated more efficient nutrient absorption (Mallikarjuna Rao et al. 1987). Increased root volume and surface area together would have led to more nutrient uptake by providing better means for greater absorption. The increased nutrient uptake due to HA would be attributed to the enhancement of microbial activity and reduced nutrient losses in the soil. The increased N uptake was supposed to be due to the better use efficiency of applied N fertilizers in the presence of humic acid coupled with retarded nitrification process enabling the slow availability of applied N (Adani et al., 1998). In addition, Inhibition of urease activity by HA (Vaughan and Ord, 1981), led to reduced losses of N by volatilization. The increase in P uptake as a result of HA and CHI application may be due to the prevention of P fixation in the soil and the formation of humophospho complexes, which are easily assimilable by the plants (Raina and Goswami, 1988) and this explains the more of P content and uptake by radish plant in the present study. The slow and continuous dissolution of phosphate minerals in soil by humic acid may account for its increased availability. Malcolm and Vaughan (1979) were of the opinion that the soil phosphatase activity improved by humic acid might have resulted in increased P availability as phosphatase hydrolyses the phosphate esters into inorganic phosphorus. The highest K uptake was recorded in the treatment receiving soil application of humic acid According to Samson and Visser (1989), humic acid induced increase in permeability of biomembranes for electrolytes accounted for increased uptake of K. Moreover, there are some reports indicate that application of chitosan increased significantly the content of potassium in plants (Farouk et al., 2008).

In conclusion, both natural chelators, in particular, chitosan at 200 mg/kg dry soil can increase the capacity of radish plant to survive under cadmium stress due to chelating the Cd in the soil, and then reduced Cd bio-availability.

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