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ALLEVIATION OF CADMIUM STRESS ON RADISH BY HUMIC ACID AND CHITOSAN AS SOIL ADDITIVES

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ABSTRACT

Two pot experiments were conducted to study the effect of humic acid (HA) or chitosan (CHI) as soil additives at concentrations of 100 and 200 mg kg^{-1} soil on counteracting the harmful effects of cadmium ions at levels of 100 and 150 mg kg^{-1} soil on radish plant. Results showed that, Cd at 100 and 150 mg kg^{-1} decreased significantly length, fresh and dry weights of shoot and root organs as well as leaves number per plant in both seasons. Chlorophyll, total sugars, nitrogen, phosphorus, potassium, relative water content, soluble proteins and total amino acids content were also decreased. Meanwhile, Cd concentration in plant tissues was increased. On the other hand, application of HA or CHI at levels of 100 or 200 mg kg^{-1} increased all the above mentioned parameters and decreased Cd concentration in plant tissues. In conclusion, both natural chelating compounds, in particular, CHI at 200 mg kg^{-1} dry soil can increase the capability of radish plant to survive under cadmium stress due to chelating Cd^{2+} ions and reducing Cd bio-availability.
Keywords: Humic acid, Chitosan, Cadmium, Radish.

INTRODUCTION

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). Due to high Cd^{2+} mobility in soil-plant system, it can easily enter the food chain and create a risk for humans, animals, plants and the whole environmental resources of our modern society (Pinto *et al.*, 2004). A part of agricultural soils all over the world is slightly to moderately contaminated by Cd due to the 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 some plant 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 plant tissues (Ramon *et al.*, 2003).

Several techniques have been investigated for their efficiency, applicability and economic feasibility for the remediation of contaminated soils. Phytoremediation, which means the use of hyperacumulator plants such as *Thlaspi caerulescens* to take up heavy metals from soils and groundwater, has revealed a 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 (Grčman *et al.*, 2001). On the other hand, EDTA revealed a vast number of negative side-effects on soil capability for plant nutrition, as it is a non-selective agent, which could chelate various 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.

Despite many studies about the effect of humic acid or chitosan on soil fertility and soil plant relationships, a little is known about their role in heavy metals remediation. Humic acid contains active acidic groups such as carboxyl and phenolic 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 soil and direct effects on physiological processes of plant (Ohta *et al.*, 2004). Chitin and chitosan are copolymers found together in nature. Chitosan has strong effects on agriculture such as acting as a carbon source for microbes in the soil, accelerating the mineralization process of organic matter and assisting the root system of plants to absorb more nutrient from the soil (Boonlertnirun *et al.*, 2008).

Therefore, objectives of this investigation are to investigate and clarify the toxic effects of cadmium on growth, yield and physiological aspects of radish plants. In addition to investigate whether the application of humic aicd or chitosan could provide a useful recovery for the adverse effects of cadmium toxicity on radish plant.

MATERIALS AND METHODS

Layout of the experiment.

Two pot experiments were carried out during two successive seasons of 2007/2008 and 2008/2009 in the greenhouse of the Faculty of Agriculture, Mansoura University in order to assess the capability of humic acid and chitosan in alleviating the harmful effect of cadmium on radish plant. The used experimental design was complete randomized block design with three replicates. Closed plastic pots (30 cm in diameter) were filled with 8 kg air dry soil, divided into three sets and contaminated with cadmium at concentrations of 0, 100 and 150 mg kg^{-1} dry soil in the form of cadmium chloride. Cadmium was added by dissolving $CdCl_2$ in the first irrigation water. In each set, pots were divided into 6 groups and treated with either humic acid or chitosan, at 100 and 200 mg kg^{-1} soil or left untreated as a control.

Soil sampling and analysis.

The type of the experimented soil was Clayey, Superactive, Mesic ، Vertic Xerofluvents. Surface soil samples (0-30 cm) were collected from the experimental field. The collected samples were air-dried, grounded and passed through a 2-mm sieve. Particle size distribution of the soil was carried out using the pipette method (Dewis and Fertias, 1970). Soil field capacity was determined by the method described by Richards (1954). Soil reaction (pH), and soil electrical conductivity (EC) were determined in the saturated soil paste, and the saturated soil paste extract, respectively, according to Richards (1954). Total carbonate was estimated gasometrically using Collin's Calcimeter and calculated as calcium carbonate according to Dewis and Fertias, (1970). Soil organic matter content was determined using Walkley & Black method as described by Hesse (1971). Amounts of water soluble cations (Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}) and anions ($CO_3{}^{2-}$, HCO_3^{-} and Cl^{-}) were determined in the extraction of saturated soil paste by the method described by Hesse (1971), whereas ($SO_4{}^{2-}$) ions were calculated as the difference between total cations and anions. Soil available nutrients (N, P and K) were extracted and determined according to Hesse (1971). Soil available nitrogen was extracted using KCl (2.0 M) and determined by using macro-Kjeldahl method. Soil available phosphorus was extracted with $NaHCO_3$ (0.5 M) at pH 8.5 and determined colorimetrically after treating with ammonium molybdate and stannous chloride at a wavelength of 660 nm. Available potassium was determined by extracting soil with ammonium acetate (1.0 M) at pH 7.0 using flame photometer. Some physical and chemical properties of the experimented soil are listed in Table (1).

Table (1): Some physical and chemical properties of the used experimental soil.

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 Cd^{2+} (mg Kg^{-1})		1.50

Cultivation process.

Twenty uniform seeds of radish (*Raphanus sativus*, L. var. sativus) were sown on 10th of April in both seasons. Irrigation was adjusted to reach the field capacity, and the assumed field capacity was readjusted every three days with the irrigation water. All of agricultural processes were carried out according to the recommendations of the Egyptian Ministry of Agriculture. Three weeks after sowing, plants were thinned to leave 5 uniform plants per pot. At harvest (45 days from sowing) , length of both the root and the shoot systems and their fresh and dry weights as well as leaves number per plant were recorded.

Plant analysis.

Chlorophyll content was extracted from the fresh samples by methanol for 24 hours at the laboratory temperature after adding traces from sodium carbonate, and determined spectrophotometrically according to Wellburn (1994). Plant samples were dried at 70°C, and ground using stainless steel equipment.

Subsamples were taken from plant shoot, and 0.2 g was digested using 5 cm^3 from the mixture of sulphuric acid and perchloric acid (1:1) as described by Peterburgski (1968). Nitrogen concentration was determined by using micro-Kjeldahl method (Cottenie *et al.*, 1982). Phosphorous was determined using ammonium molybdate and ascorbic acid (Cooper, 1977). Total potassium was determined by using Gallen Kamp flame photometer as mentioned by (Cottenie *et al.*, 1982). Cadmium concentration was determined after digesting 0.2 g from the plant dry sample by 5 cm^3 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 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 calculated according the method of Sanchez *et al.* (2004), where leaves were weighed 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 °C to determine dry weight (DW) as follows:

RWC (%) = {(FW-DW)/ (TW-DW)} x100.

Statistical analysis

Data were statistically analyzed according the 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 AND DISCUSSION

Growth parameters

Generally, severe reduction in plant growth manifested by smaller, chlorotic, wilted, and rolled leaves was recorded due to cadmium toxicity. Results in Tables 2 and 3 show that contaminated soil with cadmium at both concentrations has resulted in a significant reduction ($p<0.05$) in plant growth chractristics in both seasons. High cadmium concentration had more deleterious effects on plant growth than the low concentration. The reduction in root growth was more pronounced by Cd stress than shoot. The reduction in both radish shoot and root under cadmium stress may be attributed partially to the inhibitory effect to mitosis, reducing the synthesis of cell-wall components, and changing 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 the formation of small cells and intercellular space areas (Barcelo *et al.*, 1988).

Addition of humic acid or chitosan improved plant growth through chelating Cd ions and decreasing its availability in the rhizosphere (Pinto *et al.*, 2004). Chitosan at the level of 200 mg kg^{-1} was the superior treatment in alleviating the harmful effects of high cadmium concentration in radish plant. There are other suggested theories, which explain the role of HA or CHI on promoting plant growth. It is well established that humic substances or CHI are able to complex metal ions, which will decrease nutrients leaching with irrigation water, and increase fertilizers use efficiency (Stevenson, 1982). Chitosan has also been found to activate several biological processes of plant defense responses, such as enzymatic activities, which could be a participant in the early defense mechanisms to prevent pathogen infections (Ben-Shalom *et al.*, 2003). Furthermore, it reduces the population of fungal plant pathogens in soil, resulting in an increase in crop yield (Kobayashi *et al.*, 2002).

Chlorophyll and total sugar contents.

The effect of cadmium stress and soil chelators on chlorophylls a, b as well as total chlorophyll and total sugars concentration in radish leaves is shown in Tables 4 and 5. It is evident that, addition of cadmium ions to the soil has resulted in a significant reduction ($p<0.05$) in chlorophyll and total sugars contents. Moreover, the reduction in Chl. b was extremely sharp, which resulted in a higher Chl. a:b ratio as the concentration of Cd increased in soil (Table 4). These results are in great accordance with those obtained by Azevedo *et al.*, (2005).

The presence of soil chelators, especially CHI at the level of 200 mg kg^{-1} counteracted the adverse effect of cadmium on total chlorophyll and total sugar contents (Table 5). Moreover, chelators mixed with soil increased significantly ($p<0.05$) chl. b which resulted in a decrease in the chl a:b ratio (Table 4).

The reduction of chlorophyll content attributed to cadmium stress could be due to the inhibition of the responsible enzymes for chlorophyll biosynthesis i.e. 5-aminolaevulinic acid dehydrates and protochlorophyllide reductase (Lanaras *et al.*, 1993). Others revealed this reduction to the impairment in the supply of magnesium and iron to the leaves (Greger and Ogren, 1991). Moreover, cadmium may substitute magnesium in chlorophyll molecules (Kupper *et al.*, 1998).

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Table 5: Total chlorophyll and total sugars contents in radish leaves as affected by cadmium or chelators as well as their interaction.

Treatments	First season							
	Cadmium (A) (mg Kg^{-1})							
Chilators (B)	Control	100	150	Mean	Control	100	150	Mean
(mg Kg^{-1})	Total chlorophyll, µg g^{-1}				Total sugars, %			
0	1.696	1.176	0.895	1.255	1.977	1.403	1.117	1.449
CHI100	1.866	1.444	1.235	1.515	2.313	1.743	1.547	1.868
CHI200	2.132	1.762	1.515	1.803	2.940	2.200	1.823	2.321
HA100	1.894	1.570	1.408	1.624	2.500	1.923	1.573	1.999
HA200	2.032	1.750	1.417	1.733	2.647	2.103	1.627	2.126
Mean	1.924	1.540	1.294		2.475	1.875	1.537	
LSD 0.05	A 0.0193	B 0.0248	AB 0.0436		A 0.0332	B 0.0427	AB 0.0739	
	Second season							
0	1.675	1.165	0.904	1.248	1.877	1.273	1.030	1.393
CHI100	1.845	1.532	1.343	1.573	2.213	1.643	1.460	1.772
CHI200	2.101	1.853	1.621	1.858	2.863	2.090	1.730	2.228
HA100	1.864	1.674	1.517	1.685	2.390	1.833	1.441	1.888
HA200	2.011	1.819	1.536	1.788	2.577	1.963	1.531	2.024
Mean	1.899	1.608	1.384		2.384	1.761	1.439	
LSD 0.05	A 0.0182	B 0.0241	AB 0.0441		A 0.040	B 0.051	AB 0.089	

The Reduction in total sugars content induced by cadmium treatments may be due to its inhibitory effect on photosynthetic activities, photosynthetic pigment concentrations as well as on the activity of ribulose diphosphate carboxylase leading to a decrease in all sugar fractions (Stibrova *et al.*, 1986). The role of soil chelators in increasing chlorophylls and sugars contents under normal or polluted soil conditions may be attributed to the increasing of macro and micronutrients uptake, which increased the number of chloroplast per cell as well as photosynthetic efficiency.

Water status

Results in Table 6 indicate that increasing concentration of cadmium up to 150 mg kg^{-1} decreased significantly ($p<0.05$) water content and relative water content of radish plant as compared to the control. On the other hand, addition of soil chelators increased significantly water content and relative water content. Concerning the effect of soil additives application, results obtained that there was a significant increase in RWC and water content as a result of alleviating the harmful effect of Cd on water status of radish leaves. Once again, CHI at the level of 200 mg kg^{-1} was the superior treatment in this concern.

According to Barcelo and Poschenrieder (1990), heavy metals stress may result in a reduction in water uptake through:1) decreased root elongation, 2) decreased rate of assimilates movement from shoots to roots, 3) loss of endodermis integrity,4) increased root suberization and lignification, and 5) increased rate of root tip dieback.

Table 6: Water content and relative water content percentages in radish leavesas affected by cadmium or chelators as well as their combinations

Treatments	First season							
	Cadmium mg/Kg (A)							
Chilators (B)	Control	100	150	Mean	Control	100	150	Mean
(mg/Kg)	Leaves water content, %				Relative water content, %			
0	88.66	86.00	81.16	85.27	74.50	62.92	60.41	65.94
CHI100	89.68	87.64	86.63	87.98	79.56	69.68	64.32	71.18
CHI200	90.84	89.33	88.04	89.90	85.57	77.36	71.69	78.21
HA100	90.01	88.33	87.01	88.45	81.65	72.97	66.00	73.54
HA200	90.40	89.08	87.36	88.94	84.06	76.35	68.17	76.19
Mean	89.92	88.08	86.04		81.06	71.85	66.12	
LSD 0.05	A	B	AB		A	B	AB	
	0.2555	0.330	0.571		0.523	0.676	1.174	
	Second season							
0	88.64	85.05	81.10	84.93	73.42	82.51	63.62	73.18
CHI100	89.71	87.61	86.67	88.00	79.50	69.44	63.75	70.89
CHI200	90.78	89.33	88.08	89.40	85.49	77.32	71.42	78.08
HA100	90.08	88.36	87.05	88.49	81.38	72.72	65.49	73.19
HA200	90.36	89.05	87.39	88.93	83.24	76.06	67.52	75.60
Mean	89.91	87.88	86.06		80.61	75.61	66.36	
LSD 0.05	A	B	AB		A	B	AB	
	0.145	0.188	0.325		1.371	1.770	3.067	

On the other hand, application of both chelators increased significantly root growth represented in its length and thickness (Table 3), which increased the plants absorption ability. These results are in harmony with the results obtained by Eyheraguibel *et al.*, 2008, who attributed the high water consumption to the promotion of root growth.

Soluble proteins and total free amino acids.

Cadmium levels up to 150 mg kg^{-1} soil significantly reduced the total free amino acids and soluble protein content in the shoots of radish plants ($p<0.05$). The highest reduction was observed under high cadmium concentration (Table 7). On the other hand, application of soil chelators increased significantly both of soluble proteins and total free amino acids, and CHI at 200 mg kg^{-1} was the superior treatment. Data also indicate that application of chelators under all cadmium levels increased significantly the content of total free amino acids and total soluble proteins as compared to untreated plants under such cadmium level (Table 7).

It was reported that, ions of Cd^{2+} reduced the absorption of nitrate by about 70%, due to the inhibition of nitrate reductase activity in roots (Gouia *et al.*, 2000). Since the nitrogen content in plants treated with Cd was reduced, ultimately, amino acids and protein contents of the plants were also reduced. Similar results were reported by Hegazy (2001) on radish and faba bean plants. On the other hands, application of soil chelators counteracted the harmful effect of cadmium on total amino acid and soluble proteins due to their effect on increasing the uptake of nitrogen, which is a precursor of amino acids.

Table 7: Soluble proteins and total free amino acids content as affected by cadmium or chelators as well as their combinations

Treatments	First season							
	Cadmium mg Kg^{-1} (A)							
Chilators (B) (mg/Kg)	Control	100	150	Mean	Control	100	150	Mean
	Soluble protein content, %				Total amino acids, %			
0	3.31	2.09	1.38	2.26	4.55	3.11	2.39	3.35
CHI100	3.74	2.84	2.41	3.00	5.10	4.11	3.36	4.19
CHI200	4.75	3.53	3.03	3.77	6.02	4.99	4.27	5.09
HA100	4.01	3.17	2.52	3.23	5.35	4.42	3.65	4.47
HA200	4.25	3.43	2.69	3.46	5.64	4.82	3.92	4.79
Mean	4.01	3.01	2.41		5.33	4.29	3.52	
LSD 0.05	A 0.1039	B 0.1342	AB 0.2324		A 0.075	B 0.097	AB 0.168	
	Second season							
0	3.22	1.98	1.26	2.15	4.49	3.01	2.28	3.26
CHI100	3.61	2.84	2.27	2.91	5.00	4.00	3.25	4.09
CHI200	3.66	3.40	2.91	3.66	5.91	4.90	4.19	5.00
HA100	3.89	3.08	2.37	3.11	5.24	4.32	3.53	4.36
HA200	4.14	3.30	2.57	3.34	5.58	4.70	3.85	4.71
Mean	370	2.92	2.28		5.24	4.19	3.42	
LSD 0.05	A 0.086	B 0.111	AB 0.192		A 0.062	B 0.081	AB 0.140	

Bioaccumulation of cadmium and nutrient content.

Data illustrated in Tables 8 and 9 show that increasing cadmium concentration in soil up to150 mg kg^{-1} increased significantly the concentration of cadmium in plant tissue ($p<0.05$). Whereas the concentration of nitrogen, phosphorous and potassium were significantly decreased. On the other hand, application of humic acid or chitosan as a soil additives decreased significantly cadmium concentration in radish plant growing in the presence of cadmium, in addition to the increasing of nitrogen, phosphorous and potassium concentration as compared to untreated plants. CHI at 200 mg kg^{-1} soil was the most effective treatment in this concern.

Cadmium was reported to reduce the uptake of nitrogen, phosphorus and potassium (Narwal *et al.*, 1993). Due to its effect on plant water relationships, cadmium may lead to a direct reduction in the absorption surfaces by inhibiting the formation of root hairs, and reduceing membrane permeability (Barcelo and poschenriederi, 1990). A large increase of nutrient uptake was recorded for the application of HA and CHI. The increased nutrient availability by HA and CHI addition could be due to the enhancement of microbial activity as well as increasing root growth, which facilitated more efficient nutrient absorption (Mallikarjuna Rao *et al.* 1987).

The increased N uptake by HA and CHI application 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 (Kiss and Simihâian, 2002), may led to reduce losses of N by volatilization.

Table 8: Cadmium and nitrogen concentration in radish shoot as affected by cadmium or chelators as well as their interaction.

Treatments	First season							
	Cadmium, mg Kg^{-1} soil (A)							
Chilators (B)	Control	100	150	Mean	Control	100	150	Mean
(mg Kg^{-1})	Cadmium, mg Kg^{-1}				Nitrogen, %			
0	0.23	40.00	47.50	29.24	3.22	3.96	3.29	3.49
CHI100	0.18	15.26	25.07	13.50	4.06	3.64	3.36	3.69
CHI200	0.07	9.00	12.25	7.11	4.20	3.99	3.64	3.94
HA100	0.15	22.41	29.0	17.19	4.06	3.71	3.43	3.73
HA200	0.08	17.5	22.5	13.36	4.13	3.99	3.57	3.90
Mean	0.14	20.83	27.26		4.07	3.73	3.45	
LSD 0.05	A	B	AB		A	B	AB	
	0.004	0.56	0.97		0.075	0.096	0.167	
	Second season							
0	0.21	39.42	46.75	28.79	3.81	3.52	3.05	3.46
CHI100	0.16	14.31	24.45	12.97	3.95	3.70	3.57	3.74
CHI200	0.06	8.70	11.81	6.86	4.06	3.86	3.76	3.89
HA100	0.14	21.84	24.29	15.42	3.97	3.81	3.59	3.79
HA200	0.07	16.8	21.5	12.79	4.05	3.85	3.64	3.85
Mean	0.13	20.21	25.76		3.96	3.75	3.52	
LSD 0.05	A	B	AB		A	B	AB	
	0.005	0.660	1.143		0.028	0.039	0.068	

Table 9: Phosphorous and Potassium concentration in radish shoot as affected by cadmium or chelators as well as their combinations

Treatments	First season							
	Cadmium mg/Kg soil (A)							
Chilators (B)	Control	100	150	Mean	Control	100	150	Mean
(mg Kg^{-1})	Phosphorus, %				Potassium, %			
0	0.40	0.38	0.37	0.38	3.95	3.42	3.21	3.52
CHI100	0.41	0.39	0.38	0.39	4.00	3.85	3.65	3.83
CHI200	0.42	0.40	0.39	0.40	4.80	3.95	3.90	4.21
HA100	0.41	0.40	0.39	0.40	4.05	4.23	3.70	3.99
HA200	0.42	0.40	0.39	040	4.20	3.95	3.30	3.81
Mean	0.412	0.394	0.384		4.20	3.88	3.55	
LSD 0.05	A	B	AB		A	B	AB	
	0.0075	0.0095	NS		0.1192	0.165	0.266	
	Second season							
0	0.398	0.353	0.319	0.357	3.89	3.36	3.20	3.48
CHI100	0.402	0.394	0.387	0.394	3.98	3.82	3.69	3.83
CHI200	0.416	0.400	0.396	0.404	4.29	3.94	3.82	4.02
HA100	0.406	0.398	0.389	0.398	4.10	3.86	3.72	3.89
HA200	0.412	0.399	0.391	0.401	4.20	3.91	3.76	3.96
Mean	0.407	0.389	0.377		4.09	3.78	3.64	
LSD 0.05	A	B	AB		A	B	AB	
	0.002	0.002	0.004		0.038	0.049	0.086	

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 phosphate complexes (Larsen *et al.*, 1959), which increased phosphorus uptake. Malcolm and Vaughan (1979) supported the hypothesis that soil phosphatase activity is improved by humic acid, which may have resulted in increasing P availability as phosphatase hydrolyses the phosphate esters into inorganic phosphorus.

The highest K uptake was recorded in the treatment receiving soil application of HA or CHI . According to Samson and Visser (1989), humic acid induced an increase in the permeability of biomembranes for electrolytes accounted for increased uptake of K. Moreover, there are some reports indicate that application of CHI increased significantly the content of potassium in plants (Farouk *et al.*, 2008).

In conclusion, both natural chelators, in particular, chitosan at 200 mg kg^{-1} 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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تقليل إجهاد الكادميوم علي نبات الفجل بواسطة حمض الهيوميك والكيتوزان كإضافات أرضية

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تم إجراء تجربتي أصص لدراسة تأثير حمض الهيوميك والكيتوزان كإضافات أرضية بتركيز 100 و 200 مجم كجم$^{-1}$ في تقليل التأثير الضار للكادميوم بتركيز 100 و 150 مجم كجم$^{-1}$ تربة جافة علي نبات الفجل. أظهرت النتائج أن الكادميوم بتركيز 100 و 150 مجم كجم$^{-1}$ أدت إلي إحداث خفض معنوي في الطول وكل من الوزن الطازج والجاف للمجموع الجذري والخضري و وعدد الأوراق في كلا الموسمين. كما حدث انخفاض معنوي في قيم كل من محتوي الكلوروفيل، السكر الكلي، تركيز النيتروجين والفسفور والبوتاسيوم، المحتوي المائي، نسبة العجز المائي بالإضافة إلي كل من البروتين الذائب والمحتوي الكلي للأحماض الأمينية بينما حدثت زيادة معنوية في تركيز الكادميوم. علي الجانب الآخر أدت إضافة حمض الهيوميك أو الكيتوزان كمواد مخلبية بتركيز 100 أو 200 مجم كجم$^{-1}$ إلي زيادة كل المعايير سابقة الذكر كما حدث انخفاض معنوي في تركيز الكادميوم في أنسجة النبات. عموما كلا المادتين المخلبيتين وبصفة خاصة الكيتوزان بتركيز 200 مجم كجم$^{-1}$ ممكن أن تؤدي إلي زيادة قدرة نبات الفجل علي تحمل تحت سمية الكادميوم وذلك لحدوث خلب لأيونات الكادميوم وتقليل صلاحيته بالنسبة للنبات.

قام بتحكيم البحث

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Table 2: Fresh weight, dry weight and leaves number per plant of radish as affected by cadmium or soil chelators as well as their interaction in both seasons.

Treatments	First season											
	Cadmium (A) (mg Kg^{-1})											
Chilators (B) (mg Kg^{-1})	Control	100	150	Mean	Control	100	150	Mean	Control	100	150	Mean
	Shoot fresh weight, g				Shoot dry weight, g				Leaves number/plant			
0	34.83	21.46	16.70	24.33	3.06	1.85	0.60	1.84	6.33	4.66	3.00	4.66
CHI100	37.00	25.56	22.10	28.22	3.36	2.58	1.90	2.61	7.00	6.33	5.33	6.22
CHI200	49.93	35.66	30.06	38.55	4.15	3.31	2.64	3.37	7.00	7.00	6.33	6.77
HA100	38.20	33.66	22.40	31.42	3.44	2.75	2.04	2.74	7.00	6.33	5.66	6.33
HA200	42.66	35.00	24.86	34.17	3.84	3.16	2.46	3.15	7.00	6.33	6.00	6.44
Mean	40.52	30.27	32.22		3.57	2.73	1.93		6.86	6.13	5.26	
LSD 0.05	A	B	AB		A	B	AB		A	B	AB	
	1.35	1.74	3.02		0.126	0.162	0.282		0.415	0.534	0.929	
	Second season											
0	32.56	21.33	18.36	24.08	2.90	1.61	0.71	1.74	6.00	4.33	2.66	4.33
CHI100	36.23	26.20	23.73	28.72	3.26	2.48	1.89	2.54	6.66	6.00	5.00	5.88
CHI200	45.90	34.83	27.63	36.12	3.94	3.14	2.51	3.20	6.66	6.66	6.00	6.44
HA100	41.10	30.53	24.43	32.02	3.49	2.78	2.13	2.80	6.66	6.00	5.33	6.00
HA200	42.30	34.13	25.46	33.96	3.77	3.01	2.35	3.04	6.66	6.00	5.66	6.11
Mean	39.62	29.40	23.92		3.47	2.60	1.92		6.53	5.80	4.93	
LSD 0.05	A	B	AB		A	B	AB		A	B	AB	
	0.846	1.094	1.899		0.067	0.086	0.149		0.332	0.430	0.745	

Table 3: Shoot length, root length and root thickness of radish plant as affected by cadmium or soil chelators as well as their interaction in both seasons.

Treatments	First season											
	Cadmium (A) (mg Kg^{-1})											
Chilators (B) (mg Kg^{-1})	Control	100	150	Mean	Control	100	150	Mean	Control	100	150	Mean
	Shoot length, cm				Tap Root length, cm				Tap root thickness,cm			
0	22.0	19.4	16.7	19.37	11.6	8.76	8.06	9.47	1.540	1.050	0.663	1.08
CHI100	25.0	21.5	20.0	22.17	12.6	10.6	9.53	10.91	1.683	1.383	1.277	1.45
CHI200	26.2	24.4	21.9	24.17	13.3	11.6	10.6	11.83	1.863	1.607	1.393	1.62
HA100	25.3	22.6	21.1	23.00	13.0	11.1	10.2	11.43	1.783	1.500	1.283	1.52
HA200	25.9	24.2	21.3	23.80	13.2	11.3	10.5	11.67	1.860	1.587	1.403	1.62
Mean	24.88	22.42	20.20		12.74	10.67	9.79		1.746	1.425	1.204	
LSD 5%	A	B	AB		A	B	AB		A	B	AB	
	0.679	0.877	NS		0.513	0.662	NS		0.058	0.075	0.130	
	Second season											
0	23.13	19.36	15.43	19.31	10.93	9.33	8.33	9.53	1.53	1.05	0.67	1.08
CHI100	24.40	21.63	19.90	21.98	12.33	10.70	9.94	10.99	1.68	1.38	1.28	1.45
CHI200	25.83	23.63	21.26	23.57	13.23	11.50	10.33	11.69	1.86	1.61	1.39	1.62
HA100	25.66	21.83	21.13	22.87	12.83	10.56	10.23	11.21	1.78	1.49	1.28	1.52
HA200	25.43	23.60	21.06	23.36	13.03	11.16	10.43	11.54	1.85	1.59	1.40	1.61
Mean	24.89	22.01	19.76		12.47	10.65	9.85		1.74	1.42	1.20	
LSD 5%	A	B	AB		A	B	AB		A	B	AB	
	0.210	0.274	0.476		0.320	0.412	NS		0.059	0.076	0.141	

Table 4: Chlorophyll a and b content as well as chlorophyll a:b ratio in radish leaves as affected by cadmium or chelators as well as their interaction.

Treatments	First season											
	Cadmium (A) (mg Kg^{-1})											
Chilators (B)	Control	100	150	Mean	Control	100	150	Mean	Control	100	150	Mean
(mg Kg^{-1})	Chlorophyll A, µg g^{-1}				Chlorophyll B, µg g^{-1}				$Chlorophyll_{a:b}$ ratio			
0	1.190	0.921	0.712	0.941	0.505	0.254	0.182	0.314	2.372	3.628	3.932	3.311
CHI100	1.092	1.053	0.957	1.034	0.773	0.389	0.276	0.479	1.413	2.704	3.460	2.526
CHI200	1.080	1.121	1.092	1.098	1.051	0.642	0.422	0.705	1.029	1.766	2.600	1.798
HA100	1.065	1.106	1.057	1.076	0.829	0.464	0.350	0.548	1.285	2.381	3.025	2.230
HA200	1.139	1.131	1.053	1.108	0.892	0.618	0.363	0.624	1.281	1.845	2.914	2.013
Mean	1.113	1.066	0.974		0.810	0.474	0.319		1.476	2.465	3.186	
LSD 0.05	A	B	AB		A	B	AB		A	B	AB	
	0.020	0.025	0.045		0.025	0.033	0.058		0.149	0.192	0.332	
	Second season											
0	1.180	0.901	0.732	0.937	0.495	0.264	0.172	0.310	2.383	3.412	4.255	3.35
CHI100	1.082	1.043	0.967	1.030	0.763	0.489	0.376	0.542	1.418	2.132	2.571	2.040
CHI200	1.070	1.111	1.099	1.093	1.031	0.742	0.522	0.765	1.037	1.497	2.105	1.546
HA100	1.045	1.110	1.067	1.074	0.819	0.564	0.450	0.611	1.295	1.968	2.371	1.878
HA200	1.129	1.121	1.063	1.104	0.882	0.698	0.473	0.684	1.280	1.606	2.247	1.711
Mean	1.101	1.057	0.985		0.798	0.551	0.398		1.482	2.123	2.709	
LSD 5%	A	B	AB		A	B	AB		A	B	AB	
	0.020	0.032	0.055		0.012	0.043	0.055		0.159	0.189	0.353	