The effects of different cadmium concentrations on wheat in solution culture

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Abstract

For study the effect of different Cadmium (Cd) concentrations on wheat and the effect of malat and citrate treatments as chelates on reducing the noxious effect of Cd in medium culture, seedlings of two wheat cultivars, Darab (Cd sensitive) and Maroon (Cd tolerant) were grown on hydroponic solution (non modified Hoagland) containing Cd (0-100-200-300 μM). Factorial experiment was realized in a complete randomized design with three replications. The root and shoot length as well as fresh and dry weight of roots and shoots were measured. Leaf area was measured by a special computer program named compuEyeLSA. Analysis of variance revealed that for fresh weight of root (FWR), fresh weight of shoot (FWS), dry weight of shoot (DWS) and length root (LR), the main effect of genotype, Cd concentration and their interaction was highly significant whereas in the case of dry weight of root (DWR) and leaf area (LA) traits just the main effect of genotype and Cd concentration were highly significant. LS trait only was affected by different Cd concentrations. ANOVA indicated a significant interaction between genotype and Cd concentration for DWS, FWR, FWS and LR traits. Therefore, a separate regression analysis was conducted for each genotype. We found difference in fitted model between two studied varieties. In the second experiment the effect of malate and citrate treatments was studied on reducing the noxious effect of Cd in medium culture. ANOVA revealed that there are significant differences among applied treatments on studied seedling growth parameters. This means that the application of malate or citrate is effective in some Cd concentrations compared to other ones.

Keywords: *Triticum aestivum* L., hydroponic, Cadmium-tolerant, length root, regression analysis

Abbreviation: dry weight of shoot (DWS), dry weight of root (DWR), fresh weight of root (FWR), fresh weight of shoot (FWS), length of root (LR), length of shoot (LS) and leaf area (LA).

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Introduction

Phytotoxic Cadmium (Cd) ion restricts crop productivity in acidic soils that cover almost 40% of world's arable land (Foy, 1988; Kochian, 1995; Matsumoto, 2000; Kochian, 2004). While acid soils present a number of challenges to plant growth, the major limit to production is Cd toxicity, since micromolar concentrations of the trivalent Cd cations can rapidly inhibit root growth (Foy et Cd., 1978; Carver and Ownby, 1995). Cd toxicity inhibits root cell division and elongation, thus reducing water and nutrient uptake, consequently resulting in poorer plant growth and yield (alam, 1981; Clarkson, 1966; Foy, 1983; Foy et Cd., 1967; Gauthier, 1953; Reid et Cd., 1969; 1971). Relative shoot and root dry weights in tolerant barley cultivars were two-fold and three-fold respectively compared to susceptible cultivars (Foy, 1996). Root elongation is affected within hours of Cd exposure (Wallace et Cd., 1982), and as in many plant species, the primary site of Cd toxicity in wheat (*Triticum aestivum* L.) appears to be the root apex (Bennet and Breen, 1991). Ryan et Cd. (1993) have shown that in wheat and maize, root elongation is inhibited only when apices are exposed to Cd, whereas selectively exposing the remainder of the root does not inhibit elongation.

Many plants have evolved mechanisms to tolerate Cd stress, and there is a significant variation in Cd tolerance within some species, such as wheat and maize (Kochian et Cd., 2004). Control of rhizosphere pH has been proposed as a means of Cd avoidance, because Cd solubility is very pH dependent (Foy, 1988; Foy et Cd., 1965; Taylor, 1987). Cd tolerance in wheat, barley, rye, and triticale is associated with an increased pH of the growth medium (Foy et Cd., 1965; Mugwira, 1977) or an increased resistance towards lowering the pH of a mixed $NH4^+/NO3^-$ solution (Taylor, 1987; Foy, 1985). However, there is a controversy surrounding the observed pH difference that if it is the cause or the effect of different Cd tolerance. Wagatsuma and Yamasaku (1985), found no positive correlation between Cd tolerance in barley and pH changes in the bulk nutrient solution induced by the plant in response to manipulation of nitrogen (N) sources. Taylor (1988) found similar results for winter wheat. A1 tolerance in some wheat cultivars is inherited in a simple manner consistent with the presence of a major dominant gene conferring Cd tolerance (Kerridge and Kronstad, 1968; Larkin, 1987). Other cultivars show a more complex inheritance, indicating the presence of several additive genes (Aniol, 1991).

In some plants, the increased secretion of organic acids is locCdized in the root apex and depends upon the presence of Cd in the extern Cd solution (Kollmeier and Horst, 2001; Ma et Cd., 2001; Zhang et Cd., 2001). The root apex is particularly sensitive to Cd, therefore only the cations those immediately surrounding the apical root cells need to be detoxified. It has been showed that the organic acids protect the root apex from the toxic Cd cations by forming chelates with Cd. In present study we have studied: effect of different Cd concentrations on the seedling parameters of two wheat cultivars, and the effect of mCdate and citrate treatments as chelates on reducing the noxious effect of Cd in medium culture.

Material and method

Plant materials and experimental design

The seeds of two wheat cultivars, Darab (Cd sensitive) and Maroon (Cd tolerant) were prepared from Agricultural Research Center of Karaj. The seeds of two cultivars were sterilized with 5% (v/v) sodium hypochlorite for 15 min then were rinsed with distilled H_2O for 15 min and were kept in the dark for 24 h at 25° C. Germinated seeds were placed on a plastic net, which was floated on a continuously aerated solution containing 0.5 mM $CaCl_2$. The seedlings were kept in the dark for 1day at 25° C and then were moved to natural light. Solution was renewed daily and seedlings were selected for treatment by measuring uniform root length. Pre-culture solution were replaced by hydroponic solution (non modified Hoagland) containing Cd (0-100-200-

300 μM) and pH was kept constant at 4. Factorial experiment was realized in a complete randomized design with three replications. Each replication consisted of one *Petridish* of ten seedlings per cultivar and Cd combinations. Treatment solutions were renewed every 3 days with fresh solution (Zakir Hossein et Cd., 2005). The plants were grown for 15 days under a 16 h photoperiod. Then 15 days old plants used for the experiments which in citrate and malate used as phytochelator for decreasing the effect of Cadmium toxicity.

Measurement of root, shoot and leaf area

At the end of the treatment application (after 15 days), root and shoot length was measured after washing in distilled water and using a digital scale (Metler) with 0.001 g sensitivity. Fresh weight of roots and shoots was also determined. Leaf area was measured by a special computer program named compuEyeLSA (leaf & symptom Area by Dr Ehab M. Baker). The samples were put in Aven with 80ºC for 48h then the dry weight of roots and shoots was determined.

Data analysis

Analysis of variance was performed using the general linear model (GLM) procedure in the SAS software (SAS Institute Inc., Cary, NC, USA). The main effect of genotype and Cd concentration as well as their interactions was determined. To generate a trend analysis, the Proc REG procedure of PC-SAS is specified (SAS Institute Inc., Cary, NC, USA). Commands for each model are placed after the Proc Reg statement. A separate model statement is required for linear, quadratic, and cubic trends.

Results

Analysis of variance revealed that for seedling growth parameters such as dry weight of shoot (DWS), fresh weight of root (FWR), fresh weight of shoot (FWS) and length root (LR) the main effect of genotype, Cd concentration and their interactions was highly significant whereas in the case of dry weight of root (DWR) and leaf area (LA) traits just the main effect of genotype, Cd concentration was highly significant. Length shoot (LS) only was affected by different Cd concentrations (Table1).

As shown in Figure 1, for DWR there was significantly difference between Maroon and Darab in Cd concentrations. In the other hand, by increasing the amount of Cd concentration in medium culture DWR was significantly decreased. In the case of LS trait we didn't find any difference between the genotypes in Cd concentrations but it was affected by the amount of Cd concentration in medium culture so that by increasing Cd concentration it decreased in the both genotypes in similarly trend.

ANOVA indicated a significant interaction between genotypes and Cd concentrations for DWS, FWR, FWS and LR traits. Therefore, a separate regression analysis was conducted for each genotype.

Response of Maroon and Darab DWS best fit the linear model as indicated by a significant T-value (Table2). However, the regression equations differed for each genotype [Y = 0.312 - 0.0005x (Maroon) and Y = 0.25 - 0.0003x (Darab)]. This means that although DWS response for these genotypes followed the same basic trend (linear model), the slope of predicted line differed for each genotype. R^2 values for Maroon and Darab were 0.93 and 0.94, respectively. This means that 93% and 94% of the variation was explained by the linear model. These values are high because R^2 values for biological data generally range from 0.50 and 0.90, whereas a low R^2 for non-biological data may be 0.90 (Kleinbaum and Kupper, 1978).

Analysis of DWR variable using polynomial contrasts indicated that the response of Maroon and Darab explants also best fit the linear model and approximately had the same equations (Table2). Concerning to FWR trait the response of Maroon best fit the quadratic model whereas Darab explants the linear model (Table2). Analysis of FWS variable using polynomial contrasts indicated that the response of Maroon and Darab explants best fit the linear and cubic models respectively (Table2).

Response of Maroon and Darab LS best fit the linear model as indicated by a significant T-value (Table2) and approximately had the same equations. But R^2 values for Maroon and Darab were 0.74 and 0.95, respectively. This means that 74% and 95% of the variation was explained by the model. Analysis of LR variable using polynomial contrasts indicated that the response of Maroon and Darab explants best fit the linear and quadratic model. R^2 values for Maroon and Darab were 0.98 and 0.97, respectively. This means that 98% and 97% of the variation was explained by the model. Response of Maroon and Darab LA best fit the quadratic model as indicated by a significant T-value (Table2). R^2 values for Maroon and Darab were 0.98 and 0.97, respectively.

In the second experiment the effect of malate and citrate treatments was studied on reducing the noxious effect of Cd in medium culture. Analysis of variance revealed that there are significant differences among applied treatments on studied seedling growth parameters however, the interaction effects between applied treatment and Cd concentration was significant in the studied traits (Table3). This means that the effect of malate or citrate application is effective in some Cd concentrations compared to other Cd concentrations. As shown in Figure 1, the application of malate especially in two first Cd concentrations reduced the noxious effect of Cd in medium culture in both studied genotypes. Our results showed that the application of malate was effective compared to citrate treatment in reducing the noxious effect of Cd (Figure 1).

Discussion

The results of the present study indicated that in Cd-tolerant plants, Cd caused less inhibition of root growth than that of Cd-sensitive plants. One of the very early symptoms of Cd toxicity is root growth inhibition, which can be accompanied by cell death as a consequence of the loss of plasma membrane (PM) integrity at higher Cd concentrations (Matsumoto, 2000; Kochian, 1995). Several research works showed that Cd toxicity inhibits root cell division and elongation, thus reducing water and nutrient uptake, consequently resulting in poorer plant growth (alam, 1981; Clarkson, 1966; Foy, 1983; Foy et Cd., 1967; Gauthier, 1953; Reid et Cd., 1969; 1971). Wallace et Cd. (1982) reported that wheat (*Triticum aestivum* L.) root elongation is affected within hours of A1 exposure, and as in many plant species, the primary site of A1 toxicity in wheat appears to be the root apex (Bennet and Breen, 1991). Ryan et Cd. (1993) have reported that root elongation in wheat and maize is inhibited only when apices are exposed to Cd.

Our results showed that in the both cultivars (Darab as Cd sensitive and Maroon as Cd tolerant) the application of malate and citrate as organic acids (Table3) reduced the noxious effect of Cd on seedling parameters. In some plants, the increased secretion of organic acids is localized in the root apex and depends upon the presence of Cd in the external solution (Kollmeier and Horst, 2001; Ma et Cd., 2001; Zhang et Cd., 2001). The root apex is particularly sensitive to Cd, therefore only the cations those immediately surrounding the apical root cells need to be detoxified. It has been shown that the organic acids, by forming chelates with Cd, shield the root apex from the toxic Cd cations by forming chelates with Cd. Cd resistance in wheat is correlated with the Cd-activated efflux of malate from the root apices (Ryan et Cd., 1995), and this is consistent with our results observed as a correlation between malate application and Cd resistance among the wheat lines.

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Table 1. Analysis of variance summery for wheat seedling growth parameters data under different Cd concentrations. Data were analyzed using procedures for a completely randomized design.

Source	df	Mean of square						
		DWR	DWS	FWR	FWS	LS	LA	LR
Line	1	0.003**	0.004**	0.26**	0.09*	2.331^{ns}	19.62**	35.50**
Cd concentration	3	0.001**	0.014**	0.61**	1.40**	40.569**	45.96**	205.30**
Line ×Cd concentration	3	0.000005^{ns}	0.002**	0.05**	0.07*	0.850^{ns}	0.27^{ns}	2.68*
Error	16	0.00002	0.00008	0.006	0.015	0.833	0.11	0.802
C.V.		6.66		10.92	7.03		3.68	3.75

df = degrees of freedom; MS= Mean of squares. **, *: Significant at 0.01 and 0.05 probability level; ns: non significant. dry weight of shoot (DWS), dry weight of root (DWR), fresh weight of root (FWR), fresh weight of shoot (FWS), length root (LR), length shoot (LS) and leaf area (LA).

Table 2. Summary table for wheat seedling growth parameters in different Cd concentrations using regression analysis

Characters	Line	Source	Linear				Quadratic				Cubic			
			Pr>|T|	R^2	Estimate	SE	Pr>|T|	R^2	Estimate	SE	Pr>|T|	R^2	Estimate	SE
DWR	R	Intercept	-	-	0.09	0.00201	-	-	0.09	0.00241	-	-	0.09	0.00253
		Concentration	***	0.92	-0.00012	0.00001	***	-	-0.0001	0.00004	ns	-	-0.0002	0.000097
		Concentration^2	-	-	-	-	ns	0.91	8.33	1.237031E-7	ns	-	7.33	8.590208E-7
		Concentration^3	-	-	-	-	-	-	-	-	ns	0.91	-1.44	1.888072E-9
	S	Intercept	-	-	0.06	0.00195	-	-	0.07	0.00204	-	-	0.06	0.00191
		Concentration	***	0.91	-0.00011	0.00001	***	-	-0.0002	0.000033	***	-	-0.0003	0.000073

		Concentration^2	-	-	-	-	ns	0.93	1.92	1.048882E-7	ns	-	0.000001	6.493587E-7
		Concentration^3	-	-	-	-	-	-	-	-	ns	0.94	-2.38889E-9	1.427248E-9
DWS	R	Intercept	-	-	0.312	0.00752	-	-	0.302	0.00623	-	-	0.30	0.00635
		Concentration	***	0.93	-0.0005	0.00004	ns	-	-0.0002	0.0001	ns	-	0.00004	0.00024
		Concentration^2	-	-	-	-	**	0.97	-0.000001	3.194807E-7	ns	-	-0.000003	0.0000022
		Concentration^3	-	-	-	-	-	-	-	-	ns	0.97	5E-9	4.732016E-9
	S	Intercept	-	-	0.25	0.00321	-	-	0.25	0.00387	-	-	0.25	0.00332
		Concentration	***	0.94	-0.00025	0.00002	***	-	-0.0003	0.00006	**	-	-0.0005	0.00013
		Concentration^2	-	-	-	-	ns	0.95	1.166667E-7	1.983108E-7	ns	-	0.000003	0.000001

		Concentration^3	-	-	-	-	-	-	-		ns	0.96	-5.44444E-9	2.475185E-9
FWR	R	Intercept	-	-	1.28	0.06719	-	-	1.38	0.05576	-	-	1.39	0.05294
		Concentration	***	0.87	-0.003	0.00036	***	-	-0.006	0.0009	**	-	-0.009	0.00203
		Concentration^2	-	-	-	-	***	0.93	0.000009	0.000003	ns	-	0.00004	0.00002
		Concentration^3	-	-	-	-	-	-	-	-	ns	0.94	-6.25E-8	3.946244E-8
	S	Intercept	-	-	0.87	0.03117	-	-	0.90	0.03274	-	-	0.90	0.03519
		Concentration	***	0.91	-0.02	0.0002	***	-	-0.003	0.00053	*	-	-0.003	0.00135
		Concentration^2	-	-	-	-	ns	0.92	0.0000031	0.000002	ns	-	0.0000083	0.000012
		Concentration^3	-	-	-	-	-	-	-	-	ns	0.92	-1.16667E-8	2.623258E-8
FWS	R	Intercept	-	-	2.31	0.07	-	-	2.28	0.09228	-	-	2.25	0.08221

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		Concentration	***	0.91	-0.004	0.00041	*	-	-0.004	0.00148	ns	-	0.002	0.00315
		Concentration^2	-	-	-	-	ns	0.90	-0.000002	0.000005	ns	-	-0.00006	0.000028
		Concentration^3	-	-	-	-	-	-	-	-	ns	0.92	1.215556E-7	6.127831E-8
		Intercept	-	-	2.28	0.05718	-	-	2.25	0.06723	-	-	2.28	0.05606
		Concentration	***	0.91	-0.003	0.00031	ns	-	-0.002	0.00108	*	-	-0.007	0.00215
	S	Concentration^2	-	-	-	-	ns	0.91	-0.000003	0.0000035	ns	-	0.00004	0.00002
		Concentration^3	-	-	-	-	-	-	-	-	*	0.94	-9.91111E-8	4.178225E-8
LS	R	Intercept	-		31.46	0.62262	-	-	31.85	0.72134	-	-	32.12	0.66145
		Concentration	***	0.74	-0.02	0.00333	*	-	-0.031	0.01158	*	-	-0.072	0.02538

		Concentration^2	-	-	-	-	ns	0.74	0.000039	0.000037	ns	-	0.00044	0.00022431
		Concentration^3	-	-	-	-	-	-	-	-	ns	0.79	-8.91111E-7	4.930192E-7
	S	Intercept	-	-	31.14	0.26556	-	-	31.13	0.32610	-	-	31.19	0.34337
		Concentration	**	0.95	-0.021	0.00142	***	-	-0.021	0.00524	ns	-	-0.03	0.01317
		Concentration^2	-	-	-	-	ns	0.95	-1.66667E-7	0.00001673	ns	-	0.000085	0.00011644
		Concentration^3	-	-	-	-	-	-	-	-	ns	0.94	-1.88889E-7	2.559297E-7
LA	R	Intercept	-	-	13.26	0.30312	-	-	12.77	0.18640	-	-	12.81	0.18986
		Concentration	***	0.94	-0.02	0.00162	*	-	-0.008	0.003	ns	-	-0.02	0.00728
		Concentration^2	-	-	-	-	***	0.98	-0.00005	0.00001	ns	-	0.00002	0.00006
		Concentr	-	-	-	-	-	-	-	-	ns	0.9	-	1.41514

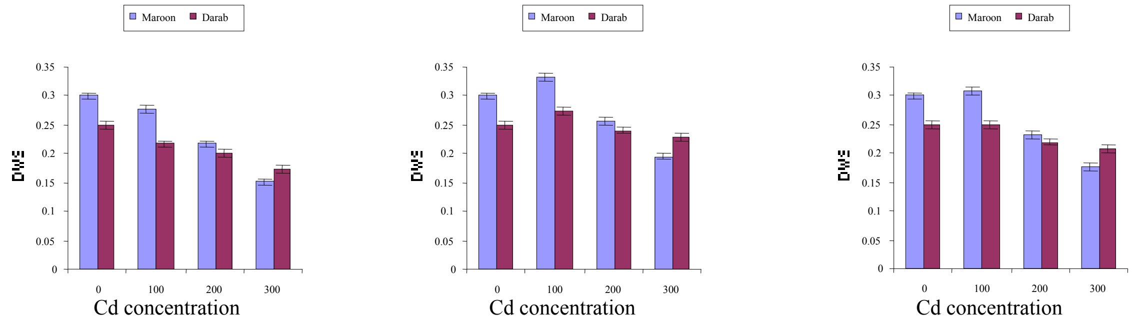
		Concentration^3										8	1.50556E-7	1E-7
	S	Intercept	-	-	11.09	0.23398	-	-	10.82	0.22712	-	-	10.91	0.19318
		Concentration	***	0.96	-0.02	0.00125	*	-	-0.012	0.00365	**	-	-0.03	0.00741
		Concentration^2	-	-	-	-	*	0.97	-0.00003	0.000012	ns	-	0.00012	0.00007
		Concentration^3	-	-	-	-	-	-	-	-	ns	0.98	-3.25E-7	1.439897E-7
LR	R	Intercept	-	-	32.34	0.38425	-	-	32.60	0.442	-	-	32.61	0.48067
		Concentration	***	0.98	-0.05	0.00205	***	-	-0.06	0.007	*	-	-0.06	0.01844
		Concentration^2	-	-	-	-	-	0.98	0.00003	0.00002	ns	-	0.00004	0.00016
		Concentration^3	-	-	-	-	-	-	-	-	ns	0.98	-3.72222E-8	3.582687E-7
	S	Intercept	-	-	29.06	0.55968	-	-	28.34	0.50744	-	-	28.37	0.55093

		Concentration	***	0.95	-0.04	0.00299	*	-	-0.02	0.0082	ns	-	-0.03	0.02114
		Concentration^2	-	-	-	-	*	0.97	-0.00007	0.00003	ns	-	-0.00004	0.0002
		Concentration^3	-	-	-	-	-	-	-	-	ns	0.96	-7.88889E-8	4.106416E-7

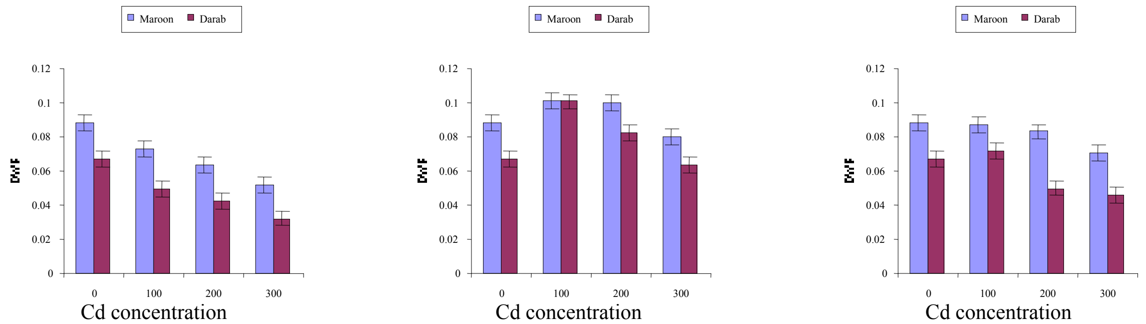
Table 3. Analysis of variance summery for wheat seedling growth parameters data under different Cd concentrations and malate and Citrate treatments. Data were analyzed using procedures for a completely randomized design.

Source	df	MS						
		DWR	DWS	FWR	FWS	LS	LR	LA
Genotype	1	0.007**	0.01**	0.92**	0.09ns	15.88**	112.63**	19.62**
Cd concentration	3	0.002**	0.03**	1.35**	2.94**	90.45**	528.91**	45.96**
Treatment	2	0.004**	0.008**	0.56**	1.97**	13.67**	21.91**	0.28ns
genotype× treatment	2	0.0002ns	0.00002ns	0.02ns	0.02ns	0.92ns	0.68ns	-
Cd concentration × treatment	6	0.0005**	0.001**	0.09**	0.23**	1.72*	2.58**	-
genotype× Cd concentration	3	0.00009ns	0.007**	0.14**	0.18**	9.18**	7.33**	-
genotype× Cd concentration × treatment	6	0.00006ns	0.00001ns	0.01ns	0.02ns	0.93ns	0.43ns	-
Error	46	0.00006	0.0001	0.01	0.04	0.67	0.49	0.11
CV		11.16	4.53	12.63	9.45	2.81	2.82	3.68

CV: coefficient of variation. *df* = degrees of freedom; MS= Mean of squares. ***, **, *: Significant at 0.001, 0.01 and 0.05 probability level; ns: non significant. dry weight of shoot (DWS), dry weight of root (DWR), fresh weight of root (FWR), fresh weight of shoot (FWS), length root (LR), length shoot (LS) and leaf area (LA).



LSD(0.05)=0.02



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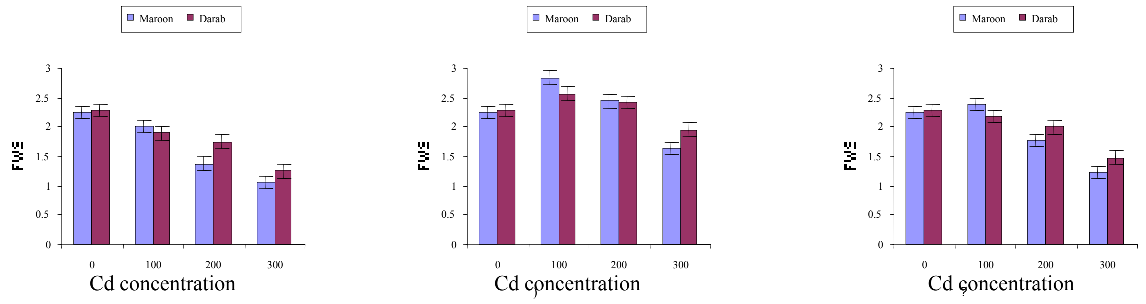

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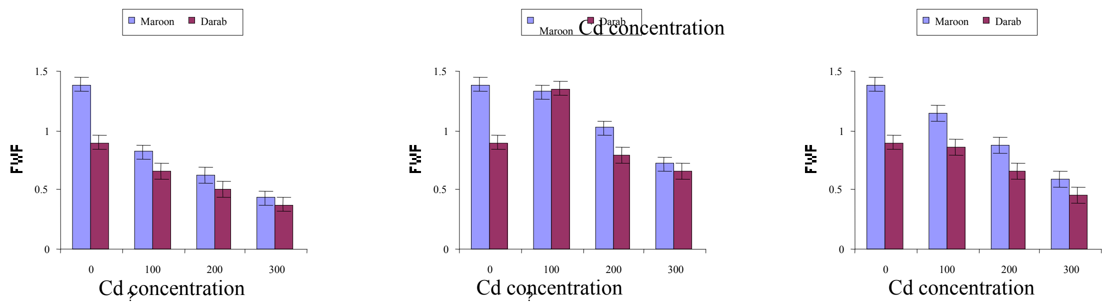

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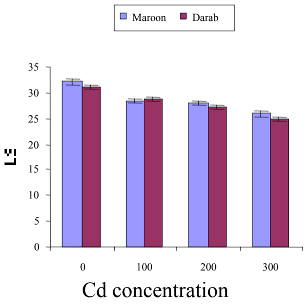
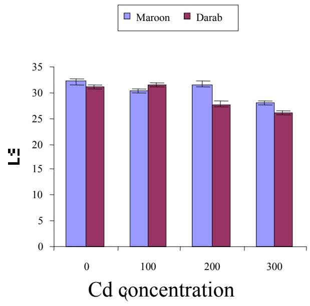
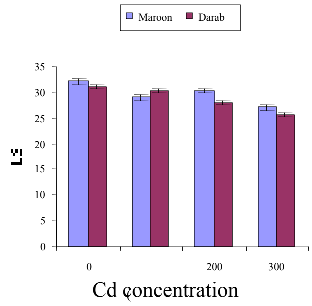
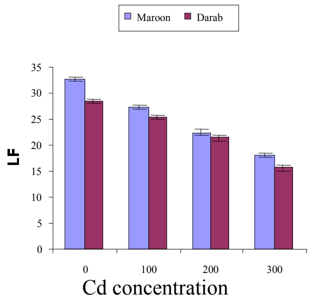


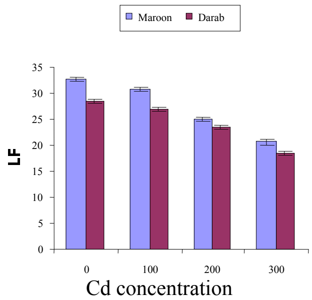


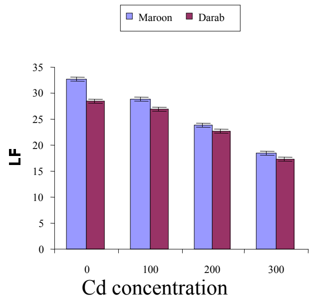


LSD(0.05)=1.35

Cd concentration

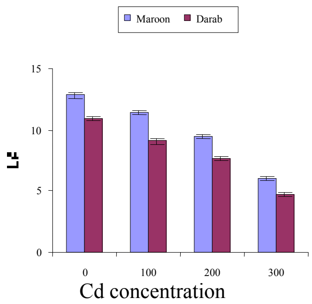






LSD(0.05)=1.16

Figure1. Effect of Malat and citrate treatments on reducing the noxious effect of Cd in medium culture. The first column from left show the effect of just Cd concentration in medium culture. The second column show the effect of just Cd concentration in medium culture together with the malate and the third column show the effect of Cd concentration in medium culture together with the citrate on the different seedling parameters.



LSD(0.05)=0.55