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ALLEVIATION THE ADVERSE EFFECT OF CADMIUM ON SEEDLING GROWTH OF GREATER BURDOCK (*ARACTIUM LAPPAL* L.) THROUGH PRE-SOWING TREATMENTS

SUMMARY

Cadmium (Cd) is one of the most important heavy and highly toxic metal elements which is very stable in environment. Its level increasing in soil brings a wide range of adverse effects on plants, especially during seed germination and seedling growth. *Arctium lappa* belongs to the Asteraceae and is an important medicinal plant of temperate regions. In this investigation, impact of pre-sowing treatments (control, osmopriming with PEG and priming with salicylic acid) on germination and seedling growth of burdock were evaluated under four concentrations of Cadmium (0, 10, 25 and 50 mg/L). The results showed that germination and seedling growth of burdock were inhibited significantly by the cadmium concentrations upper than 10 mg/L, especially in non-primed-control and osmoprimed seeds. Priming with salicylic acid is improved significantly some traits such as germination percentage, germination index and seedling early growth, as compared with control and osmopriming. Afterward burdock seedlings which experienced pre-sowing treatments were grown under hydroponic culture in presence of Cd (0-50 mg). Analysis of leaf tissue of 30-day-seedling revealed that catalase (CAT) and guaiacol peroxidase (GPX) activity enhanced by increasing of Cd concentration. However enzymatic scavenging was not sufficient for reactive oxygen detoxification, so that cadmium application increased hydrogen peroxide production and significantly was induced loss of cell membrane integrity. Decrease in total chlorophyll and soluble protein content was observed under Cd treatments. However, seedlings obtained from salicylic acid-primed seeds showed a better performance compared to other pre-sowing treatments under both control and Cd stress.

Keywords: Antioxidant, heavy metal, medicinal plant, Priming, salicylic acid

INTRODUCTION

Industrial revolution and quick population rise especially in developing countries has been faced the food security with serious challenges. Application of urban and industrial sewage for crop irrigation, increased use of fossil fuels and the return of combusted compounds to environment and the excessive use of pesticides on farms have increased the risk of heavy metals accumulation in soils

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(Cheng et al., 2002). This phenomenon is also apparent around of the large and industrial cities. Furthermore, ore mining and smelting, atmospheric precipitation and application of phosphorus fertilizers can cause heavy metal contamination.

Between all toxic heavy metals, cadmium (Cd) is one of the most hazardous environmental contaminants which can prevent or restrict plant growth (Alloway, 1994). This element is not essential for plant growth and its mobility in soils and plants is very high, hence Cd can easily enter to plant and affects growth and metabolic process (Das et al., 1997). Cadmium is very reactive and has high tendency to the functional groups (amine, carboxyl, phosphate and thiol groups) of bimolecules (Mendoza-Cózatl, 2005). High concentrations of Cd may indirectly lead to oxidative stress in plants. Therefore stimulation and increase of the antioxidant enzymes activity can be effective in heavy metal tolerance in different plants (Xu et al., 2008). Moldovan and Moldovan (2004) reported Cd can induce the generation of reactive oxygen species (ROS), which disturb cellular redox hemostasis, inactivate enzymes, and cause a lipid peroxidation and electrolyte leakage. The antioxidative enzyme system like as guaiacol peroxidase (GPX) and catalase (CAT) which participate in the decomposition of H_2O_2 may protect plants from oxidative stress caused by various heavy metals.

Salicylic acid (SA) is a phenolic compound which acts as an imperative signal molecule and is involves in plant growth regulation (Alvarez, 2000). Salicylic acid (SA) is believed to be as a hormone-like substance which provides protection against abiotic stresses and also it probably plays an important role in plant pathogenesis. It is recognized that salicylic acid can be involved in plant protection in metalliferous soils. Exogenous application of SA on seed or plant alleviated the damaging effects of heavy metals on seedling early growth of rice (Choudhury and Panda, 2004; He et al., 2010) and in barley (Metwally et al., 2003).

Seed germination is one of the most critical stages of plant development which is strongly inhibited by heavy metals of soil (Lamhamdi et al., 2011). When seed hydration is occurred under desired situation seed dormancy can be removed and metabolic system will be activated and subsequently essential biochemical processes for seedling growth occur (Sfaxi-Bousbih et al., 2010). It seems that pre-sowing seed treatments, which during those seeds hydration are done outside of field and under controlled condition, can alleviate adverse effects of abiotic stresses on seed germination and increase chance of seedling establishment (Anuradha and Rao, 2007; Farhoudi et al., 2011). Accordingly, Sharama et al. (2010) found that hormone-priming could significantly reduce toxic effects of cadmium on germination of radish (*Raphanus Sativus* L.) seeds in polluted soils. Priming is referred to the pre-sowing treatment that involves exposure of seeds to controlled hydration. This hydration is sufficient to permit pre-germinative metabolic events but insufficient to allow radicle protrusion through the seed coat.

Greater Burdock (*Aractium Lappal* L.) is one the most important medicinal plants from Asteraceae family and is found in waste places and disturbed soils.

Burdock used as a diuretic, antipyretic, blood purifier, cure for heumatism and gonorrhea, to stimulate bile and to cause long lasting reduction of blood sugar (Foster and Duke, 1990; Lin et al., 2002; Chauhan, 1999). Irregular germination could be considered as a major problem for producing medicinal plants and this may resulted from several factors, including a high proportion of non viable, unfilled or immature seeds or dormancy. Although, beneficial effects of pre-sowing treatments on germination percentage in *A. lappa* has already investigated under optimal condition (Bhardwaj et al., 2010) but the effectiveness of these treatments has not been evaluated under heavy metal stress conditions. This study was conducted to assess the effects of pre-sowing seed treatments on the germination, seedling growth and biochemical parameters of Greater Burdock under cadmium stress.

MATERIAL AND METHODS

Seeds of Greater Burdock (*Aractium Lappal* L.) were provided by local farmers of Isfahan (Iran) and their initial germination percentage was 96%. Prior to germination, the seeds were surface sterilized with 5% (v/v) sodium hypochlorite for 10 minutes and then washed with distilled water for three times. In order to study the effect of pre-sowing treatments and cadmium (Cd) stress on Greater Burdock early growth two experiments were conducted. Experiment 1 was carried out to determine the effect of the seed priming and Cd on the germination characteristics of *A. Lappal*. Experiment 2 investigated the effects of different Cd concentrations and pre-sowing treatments on antioxidants activity, total protein concentration, hydrogen peroxide concentration, cell membranes integrity and total chlorophyll in leaves of Greater Burdock grown under hydroponic culture.

Pre-sowing treatments were including control (non primed seeds), osmopriming (soaking seeds in -1MPa osmotic solution of Poly ethylene glycol-6000 for 36 h) and priming with salicylic acid (soaking seeds in 0.2 Mm salicylic acid). After presoaking, both primed and non-primed (control) seeds were washed with distilled water then allowed to air dry for 48 h at 25 ºC (Iqbal and Ashraf, 2007). Primed and unprimed seeds were grown under four level of Cd stress including of 0, 10, 25 and 50 mg/L.

Seed germination tests

A laboratory experiment was conducted using 3 pre-sowing treatments (control, osmoprimng and priming with salicylic acid) and 4 Cd concentrations (0, 10, 25 and 50 mg/L) according to 4 × 3 factorial laid out based on a randomized complete block design (RCBD) with 3 replicates. Seed germination was tested on filter papers placed in Petri dishes and moistened with 8 ml of different Cd solutions. Twenty five seeds were placed in each dish and incubated in the dark at 25 ± 1 °C and germinated seed were recorded daily for nine days. Mean germination time (MGT) was computed according to Ellis and Roberts (1981) as $MGT=\sum Ti\ Ni/\sum Ni$, where N*i* is the number of newly germinated seeds

at time T*i*. The germination index (GI) which expressed as speed of germination was calculated as GI=∑(G*t*/T*t*), where G*t* is the accumulated number of germinated seeds on day *t*, and T*t* is the time corresponding to G*t* in days (Hu *et al*. 2005). Mean shoot and root lengths at the time of harvest were measured per replication. Dry weight of seedlings were taken with the help of an electric balance after drying each replication at 70 °C in the oven to get the constant weight.

Hydroponic culture experiment

For investigating the effects of Cd on some biochemical characteristics during seedling growth, the primed and unprimed seeds were germinated in Petri dishes moistened with distilled water. After 10 days, seedlings with uniform size were selected and planted onto plastic tubs which covered by a thin layer of polystyrene and containing half-strength Hoagland nutrient solution. They were grown in a growth room (approximately 14h light/10 h dark) providing white fluorescent light and natural light with an irradiance of 250 $\mu mol.m^{-2}.s^{-1}$, day/night temperature of 25±2°C/15±2°C and 60±5% relative humidity. The Hoagland nutrient solution was consisted of (mg/L): Ca $(NO_3)_2 \cdot 4H_2O$ 945, KNO_3 607, $NH_4H_2PO_4$ 115, $MgSO_4 \cdot 7H_2O$ 493, $FeSO_4 \cdot 7H_2O$ 13.9, Na_2-EDTA 18.65, H_3BO_3 2.86, $MnSO_4 \cdot 4H_2O$ 2.13, $ZnSO_4 \cdot 7H_2O$ 0.22, $CuSO_4 \cdot 5H_2O$ 0.08, and $(NH_4)6Mo_7O_{24} \cdot 4H_2O$ 0.02. The pH of the solution was adjusted to 6.0 by dilute HCl or NaOH solution and nutrient solution was change every 10 days. After 10 days, twenty-day old seedlings were treated with Hoagland's solution containing 0, 10, 25 and 50 mg/L Cd and maintained for 10 days in these conditions. Leaves of thirty-day old seedlings were harvested and analyzed.

Enzyme and H_2O_2 essay

For CAT and GPX extraction, leaf samples (0.5g) were homogenized in ice cold 0.1 M phosphate buffer (pH=7.5) containing 0.5 mM EDTA with pre-chilled pestle and mortar. Each homogenate was transferred to centrifuge tubes and was centrifuged at 4°C in refrigerated centrifuge for 15 min at 15000×g. The supernatant was used for enzyme activity assay (Esfandiari et al. 2007) and CAT activity was measured according to Aebi (1984). About 3 ml reaction mixture containing 1.5 ml of 100 mM potassium phosphate buffer (pH=7), 0.5 ml of 75 mM H_2O_2, 0.05 ml enzyme extraction and distilled water to make up the volume to 3 ml. Reaction started by adding H_2O_2 and decrease in absorbance recorded at 240 nm for 1 min. Enzyme activity was computed by calculating the amount of H_2O_2 decomposed. Guaiacol peroxidase (GPX) was determined by measuring the oxidation of guaiacol. The assay mixture contained 10 mmol/L potassium phosphate (pH 6.4), 8 mmol/L guaiacol, and 2.75 mmol/L H_2O_2. The increase in absorbance was recorded at 470 nm within 2 min (linear phase) after the addition of H_2O_2 (Huang et al. 1996). The content of H_2O_2 was determined using the method described by Alexieva et al. (2001).

Determination of cell membrane integrity

Leaf membrane stability index (MSI) was measured as ion leakage according to Sairam et al. (2002). Fresh leaves (0.25 g) was cut into 20mm sections and placed in tubes containing 5ml of distilled water in two sets. One set was kept at 40 °C for 30 min and another set at 100 °C in Benmary bath for 15 min and their respective electric conductivity C_1 and C_2 were measured by pH/conductivity meter. The MSI was calculated as $[1-(C_1/C_2)] \times 100$.

Estimation of Protein and chlorophyll content

Soluble protein content was estimated by the method of Bradford (1976). Protein concentration is determined by quantifying the binding of the dye, Coomassie Brilliant Blue G-250, to the unknown protein solution, as compared to known standards. Tubes containing 100 µl aliquots of known concentrations of Bovine Serum Albumin (BSA; 0.156 mg l^{-1} to 10 mg l^{-1} in 0.15 M NaCl), were prepared. Also, blank tubes containing 100 µl of 0.15 M NaCl were prepared. One ml Coomassie Brilliant Blue solution was added to each tube and the mixtures vortexed. The reactions were left at room temperature for 2 min. The absorbance at wavelength of 595 nm was determined against the blank and the standard curve of absorbance versus protein concentration was plotted (Copeland 1994). Reactions containing dilutions of the soluble protein extracts (unknown concentrations) were set up as above and the absorbance at 595 nm was determined. The protein concentration of the extracts was determined from the standard curve, using Spectrophotometer.

The chlorophyll was extracted in dimethyl sulphoxide according to Hiscox and Israelstam (1979). Fresh leaves (100 mg) were kept in the extraction reagent, dimethyl sulphoxide (DMSO). The tubes were kept in the oven at 60°C for 45 min. 1 ml aliquot was mixed with 2 ml DMSO and vortexed. Then chlorophyll content was determined Spectrophotometrically. All measurements were replicated three times in independent experiments and the determination of enzyme activity, protein and chlorophyll concentration was performed with three parallel samples in all cases. Data were analyzed by two-way variance analysis using SPSS (15.0) and comparisons among means of factors combinations were examined based on the least significance difference (LSD) test at $\alpha = 0.05$.

RESULT AND DISCUSSION

Seed germination and seedling early growth

The germination percentage (GP) and germination index (GI) were decreased significantly by Cd while MGT increased by heavy metal stress (Table 1). The lowest GP was recorded at osmoprimed seeds under the highest concentration of Cd (50mg). However, under this level of Cd, the germination percentage of salicylic acid (SA) primed seeds was near to 2-folds of osmoprimed seeds. When SA-priming was compared with the other priming treatments, it was revealed that seeds soaking in salicylic acids solution significantly increased GP and GI especially under heavy metal stress (Table 1).

Table 1. Changes in germination characteristics and seedling growth of Great Burdock *Aractium Lappal* L.) as affected by seed priming treatments under different level of cadmium stress

Priming treatments	Cd (mg)	GP	RL (mm)	SL (mm)	MGT (day)	GI	SFW (g)	SDW (g)
Control	**0**	94.30	31.3	24.22	4.51	10.18	4.421	0.854
	10	83.90	23.41	15.74	5.28	9.24	3.217	0.472
	25	51.62	9.67	5.69	6.35	6.53	1.018	0.217
	50	27.63	4.48	3.22	7.76	2.51	0.761	0.104
Osmopriming	**0**	97.21	24.21	17.65	4.30	12.37	5.370	0.979
	10	82.04	19.73	11.24	5.04	8.26	4.571	0.681
	25	36.21	5.42	3.65	5.73	5.41	2.821	0.421
	50	17.34	3.49	2.76	6.74	1.70	0.653	0.183
Hydropriming	**0**	97.50	41.23	29.38	3.58	13.47	5.980	1.035
	10	86.25	28.93	19.61	4.07	10.95	3.427	0.736
	25	63.21	14.31	7.69	4.97	8.26	3.631	0.579
	50	42.17	6.22	4.21	5.26	3.54	1.021	0.165
LSD at 5%	—	*12.36*	*4.59*	*3.71*	*1.09*	*2.74*	*1.64*	*0.22*

Abbreviations, GP: germination percentage, RL: root length, SL: shoot length, MGT: mean germination time, GI: germination index, SFW: seedling fresh weight, SDW: seedling dry weight.

The highest MGT was observed in non-primed seeds under severe Cd stress. SA-priming and osmoriming could decrease MGT under all levels of Cd, but SA-priming effect was more prominent. Root and shoot length were strongly reduced with increasing cadmium concentrations. However, comparison of seed treatments showed that cadmium increase caused more severe reduction in seedling length of osmoprimed seeds. In spite of the shortest root and shoot was generated by osmoprimed seeds, SA-priming significantly increased seedling length compared to the control (Table 1). Changes of seedling fresh weight between the treatments approximately were similar to seedling length and seed soaking in salicylic solution could increase seedling fresh weight especially under higher level of Cd. The promotion of growth in Greater Burdock seedlings by SA under Cd conditions not only was associated with enhanced seedling dry weight but also increased total water in seedlings. Seedlings of non-primed seeds had the lowest both fresh and dry weight (Table 1).

CAT and GPX activity and H_2O_2 concentration

Analysis of antioxidant enzymes activity indicated that increase of Cd concentration significantly induced CAT activity (Figure 1). In the seedlings which obtained from osmoprimed seeds CAT activity at 25 mg of Cd were slightly higher but comparable with the SA-primed ones. This status was according to findings of Bailly et al. (2000), who reported considerable increase in atioxidants activity in sunflower seedling by osmotreatment.

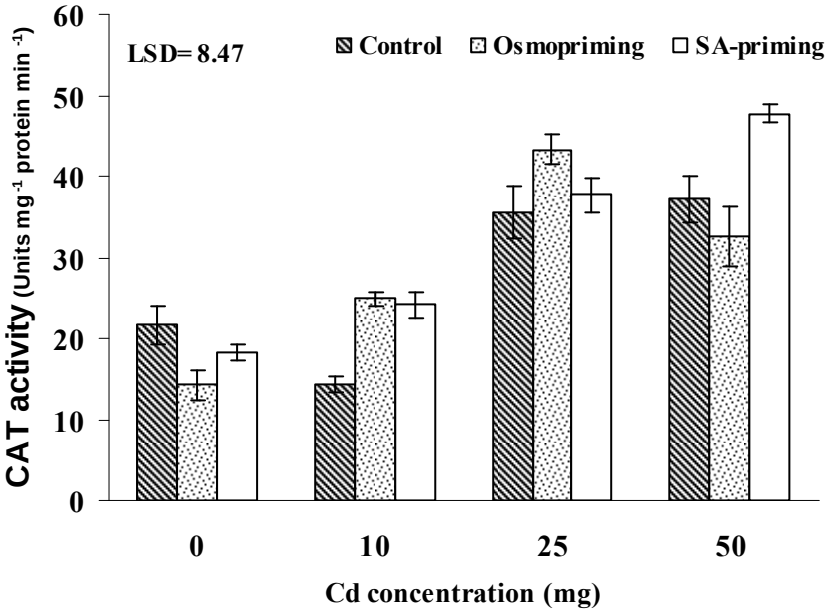

Figure 1. Effect of seed pre-sowing treatments and Cd stress on catalase activity in leaves of *Aractium Lappal* L.seedling. The values and standards errors (vertical bars) of three replications are shown.

However in seedlings of osmoprimed seeds a decrease was noticed under the highest level of Cd and in control-non-primed seedling there was not significant difference between high levels of Cd concentration (25 and 50 mg/L). Nevertheless in seedlings from SA-primed seeds CAT activity increased in regular pattern by increase of Cd concentration.

The activity of GPX in control and SA-primed seedlings showed a sharp and regular increase with increase in metal concentration, but in the case of osmopriming GPX activity decreased at the highest Cd concentration (Figure 2). Under non-stress and mild Cd stress conditions seedling obtained from SA-primed seeds showed higher GPX activity as compared to the other pre-sowing treatments.

The effect of different concentrations of Cd and pre-sowing treatments on H_2O_2 concentration is depicted in Figure 3. Hydrogen peroxide concentration significantly enhanced in all pre-sowing treatments with increasing the severity of metal stress. However it should be noted that although in seedling obtained from control-non-primed seeds H_2O_2 concentration sharply increased by increasing of Cd concentration, H_2O_2 concentration in seedling obtained from SA-primed and osmoprimed seeds did not statistically change from Cd level of 25 mg/L to 50 mg/L.

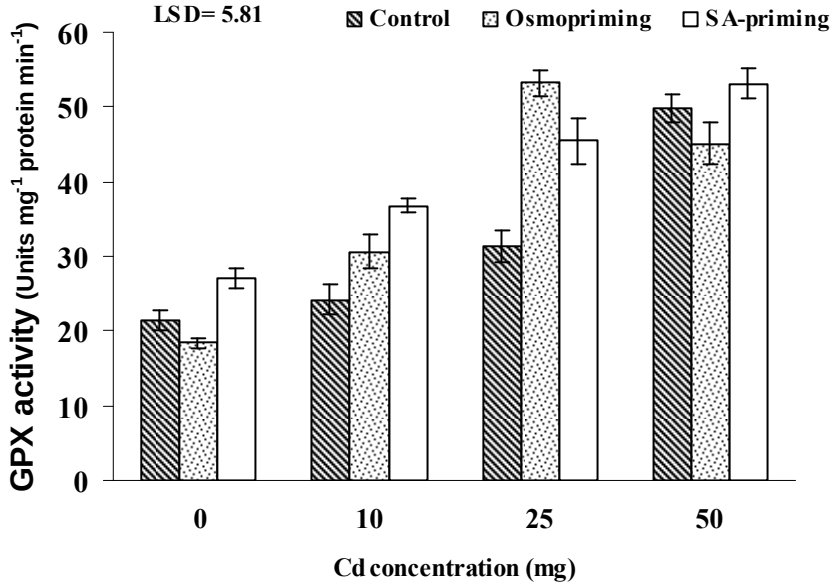

Figure 2. Effect of seed pre-sowing treatments and Cd stress on guaiacol peroxidase activity in leaves of *Aractium Lappal* L.seedling. The values and standards errors (vertical bars) of three replications are shown.

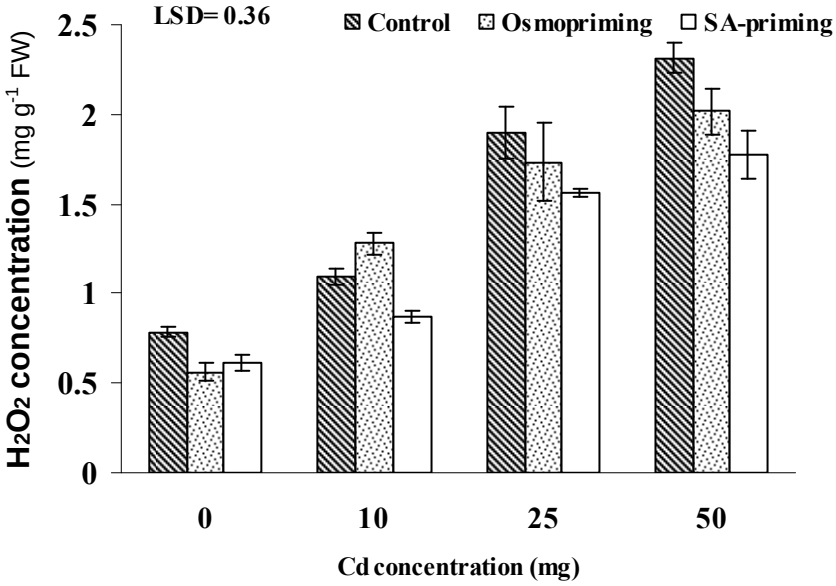

Figure 3. Effect of seed pre-sowing treatments and Cd stress on hydrogen peroxide concentration in leaves of *Aractium Lappal* L.seedling. The values and standards errors (vertical bars) of three replications are shown.

Membrane stability index

Integrity of the cell membrane was assessed indirectly by investigating solution conductivity which illustrates electrolyte leakage from cells. The data regarding MSI showed a significant effect of Cd and pre-sowing treatments (Figure 4). High concentrations of Cd, i.e. 25 and 50mg/L, drastically reduced MSI in comparison with non-stress conditions. However under mild Cd stress (10 mg/L) cell membrane integrity statically was similar to control, but at the highest concentration of heavy, MSI was reduced about 37% as compared with control. Between all pre-sowing treatments SA-priming positively influenced the MSI only under highest Cd concentration, since in seedling from SA-primed seeds the lowest membrane damage was recorded.

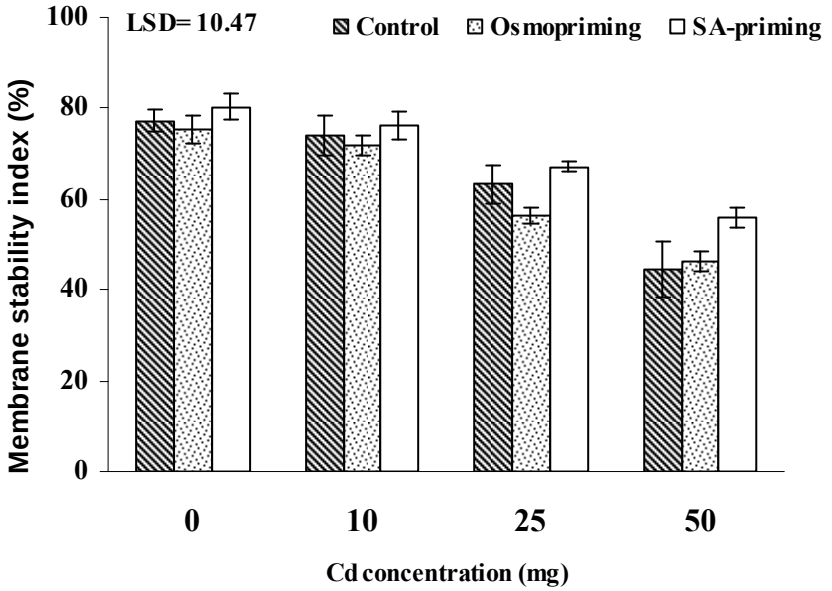

Figure 4. Effect of seed pre-sowing treatments and Cd stress on membrane stability in leaves of *Aractium Lappal* L.seedling. The values and standards errors (vertical bars) of three replications are shown.

Soluble Protein Content

The results related to the effect of Cd on soluble protein content are depicted in Figure 5. It was revealed that the trends of protein content alternation under levels of Cd were dissimilar in different pre-sowing treatments. Although, Cd treatments declined the soluble proteins in seedlings obtained from control-non-primed seeds, the mild Cd stress (10 mg/L) significantly enhanced proteins content in seedling obtained from SA-primed and osmoprimed seeds. It is noteworthy that under non-stress condition the highest protein content was recorded in seedlings obtained from control-non-primed seeds.

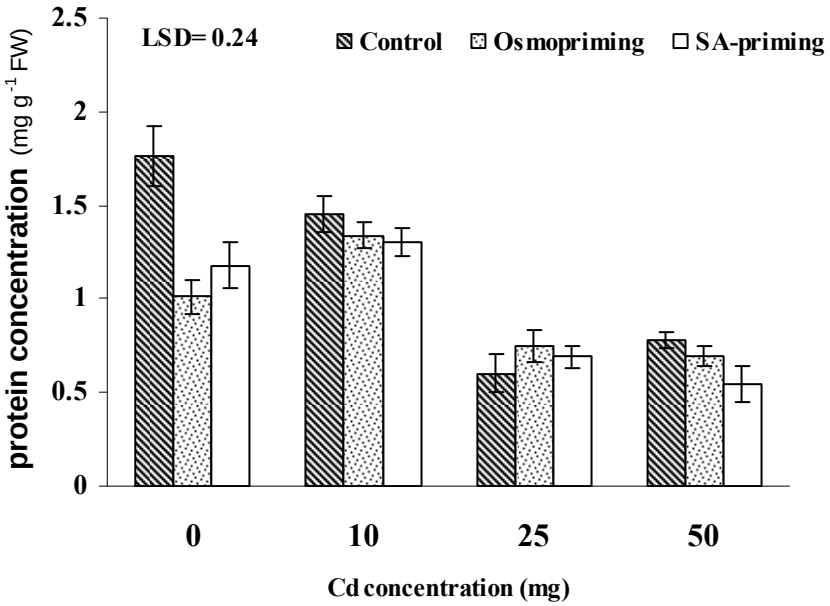

Figure 5. Effect of seed pre-sowing treatments and Cd stress on total protein concentration in leaves of *Aractium Lappal* L.seedling. The values and standards errors (vertical bars) of three replications are shown.

Chlorophyll Content

It was observed that Cd stress constantly declined chlorophyll content and the maximum chlorophyll content was recorded from non-stress condition (Figure 6). Chlorophyll content in seedlings grown under 10, 25 and 50 mg/L of Cd showed 16%, 39% and 53% reduction when compared with control, respectively. However, statistical analysis of data revealed that pre-sowing treatments could not significantly affect chlorophyll content.

In the current study, the most important sign of Cd toxicity was found to be the inhibited seed germination. Our studies about the seed germination parameters were in concordance with the findings of Al- Rumaih et al., (2001), who reported $CdCl_2$ adversely influenced the germination process of *Vigna unguiculata* seeds. Cd may adversely affect the activities of alpha-amylase and invertases in embryonic axis and cotyledon, resul in restricted mobilization of carbohydrates (starch, soluble sugars, sucrose, glucose, and fructose) for germination and embryo growth (Sfaxi-Bousbih et al., 2010).

Present study indicated that Cd toxicity was associated with the seedling growth inhibition and reduction in biomass production even so under mild Cd stress considerable reduction in seedling biomass was recorded. The reduction of seed germination under Cd stress can be attributed in part to alterations of selective permeability of cell membrane (Nouairi et al., 2006).

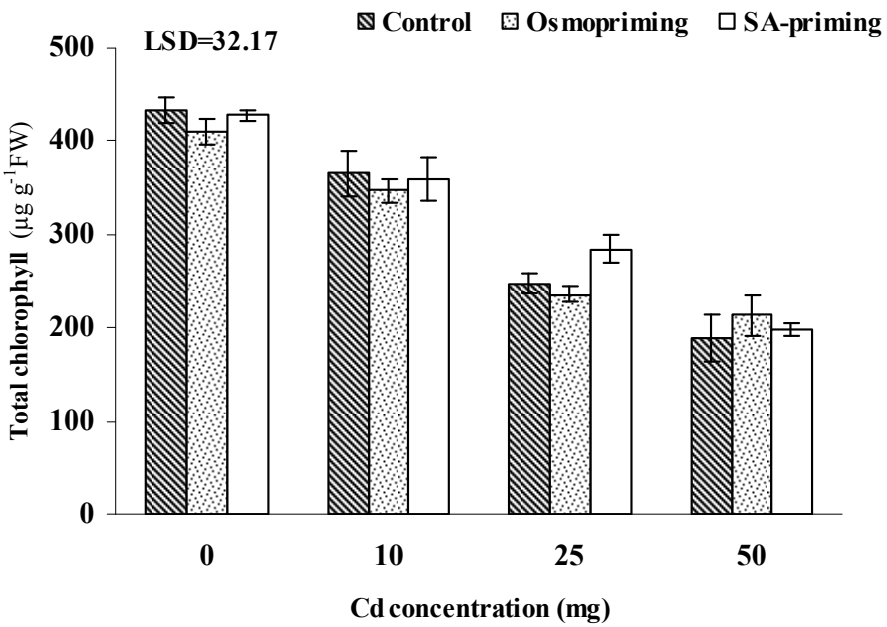

Figure 6. Effect of seed pre-sowing treatments and Cd stress on total chlorophyll concentration in leaves of *Aractium Lappal* L. seedling. The values and standards errors (vertical bars) of three replications are shown.

Reduction in biomass production under metal stress is likely caused by prevention of cell division and elongation that could be due to irreparable inhibition of some responsible proton pumps (Liu et al., 2004). Cadmium may adversely affect cellular redox hemostasis and through it reduce root growth (Schützendübel et al., 2002); therefore, reducing root growth rate may affects the uptake of water and nutrients, and this influences growth of the entire plant. The increase of seed germination under Cd stress by SA-priming compared to other treatments is probably related to satisfactory hydration. Sufficient hydrated and standard desiccated seeds can undergo many biochemical reactions if the initial hydration of proteins, in particular enzyme proteins, has taken place (Bewley and Black, 1994). In accordance with our results, seed priming with salycilic acid has been shown to enhance seed germination and seedling growth under Cd stress in rice (He et al., 2010; Choudhury and Panda 2004).

Our results also revealed that Cd stressed environment had remarkable decreased membrane integrity. This increase significantly was higher in SA free seedlings under severe Cd stress. Such an increase is reported in rice under Cd stress (Choudhury and Panda 2004) and *Arabis paniculata* under Zn stress (Zeng et al., 2011). In living organisms, plasma membrane is primary site which sense environmental stresses and may be regarded as first target for heavy metal toxicity. Lipid peroxidation is process by which the integrity of the cell

membrane is affected under heavy metal stress. Reactive oxygen species and lipooxygenases can initiate lipid peroxidation and cause membrane integrity loss (Vranova et al, 2002). It seems that protection of plasma membrane against Cd-induced damages and avoidance of solute leakage from cells may be achievable via the ROS scavenging.

Heavy metal stress is known to be trigger oxidative stress in plant tissue through the increase in reactive oxygen species (Choudhury and Panda 2004). Activity of scavenging enzymes such as CAT and GPX could remove ROS and determines the steady-state levels of ROS and other free radicals in cell (Esfandiari et al. 2007). Consequently, increase in activities of scavenging enzymes could improve stress tolerance in plants. In present study, changes in antioxidants activity were similar under all pre-sowing treatments in different levels of Cd, but seedling from SA-primed seeds showed a superior performance for H_2O_2 scavenging. This status likely caused to higher membrane stability and lower H_2O_2 concentration in seedling obtained from SA-priming in comparison with control and osmopriming. Although the increase in CAT and GPX activity were induced by metal stress, these ROS detoxification systems were not enough efficient, thus increased the chance of their accumulation in leaves tissues. However, in addition to antioxidant enzyme non-enzymatic antioxidants (e.g glutathione, ascorbate, falvonoids), Heat Shock Proteins, metallothioneins, phytochelatins (e.g. amino acids, organic acids, peptides) may contribute in heavy metal detoxification (Hall, 2002).

Our findings about the decrease in chlorophyll content under Cd stress were consistent with findings of other researcher in bean (Zengin and Munzuroglu 2005) and *Lemna polyrrhiza* (John et al 2008). The decline in chlorophyll content in plants may be due to (i) inhibition of imperative enzymes involved in chlorophyll biosynthesis (Van Assche and Clijsters 1990), (ii) disturbance in the supply of Mg^{2+} and Fe^{2+} needed for the synthesis of chlorophylls and (iii) the replacement of Mg^{2+} ions associated with the tetrapyrrole ring of chlorophyll molecule with other metals (John et al 2008). The loss in chlorophyll content can accordingly result in inhibition of photosynthesis.

Protein variation is an essential part of plant response to environmental stress as well as for adaptation to environmental conditions. Stress condition can reduce a synthesis of some proteins and up-regulate others, but in general, with increasing stress level shows a descending trend (Vierstra 1993). Our results in agreement with John et al (2008) who reported a sever decrease in protein content in *Lemna polyrrhiza* under cadmium and lead stress. Reduction in protein concentration may be resulted from increased protein degradation process through protease activity that is found to enhance under stress conditions (Vierstra 1993).

CONCLUSION

Collectively, this experiment indicate that seed priming with salicylic acid, at least in part, improved seed germination and seedling growth under Cd stress. It was demonstrated by the increase in CAT and GPX activity and lower reduction in MSI and chlorophyll content. Response of CAT, GPX and H_2O_2 revealed that oxidative stress is one the consequences of Cd stress. However, scavenging capacity of Greater Burdock was not sufficient for H_2O_2 detoxification. The tolerance of SA-primed seedling to Cd stress seems to be related their lower ion leakage and membrane damage than other pre-sowing treatments.

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UBLAŽAVANJE NEGATIVNOG DEJSTVA KADMIJUMA NA PORAST SIJANACA ČIČKA (*Aractium Lappal* L.) PREKO PREDSJETVENIH TRETMANA

Kadmijumom (Cd) je jedan od najvažnijih teških metala, pri tom vrlo otrovnih, a vrlo postojanih u spoljašnjoj sredini. Njegov nivo u zemljištu se povećava utičući višestruko negativno na biljke, posebno tokom klijanja sjemena i porasta sijanaca. Čičak pripada familiji Asteraceae i važna je ljekovita biljka umjerenog klimata. U ovom istraživanju, uticaj predsjetvenih tretmana (zaštita, podešavanje osmotske vrijednosti sa polietilen glikolom PEG i tretiranje sa salicilnom kiselinom) na klijavost i porast sijanaca čička su ocijenjivani pri četiri koncentracije kadmijuma (0, 10, 25 i 50 mg / L). Rezultati su pokazali da su klijavost i porast sijanaca čička bili značajno inhibirani kadmijumom u koncentracijama višim od 10 mg / L, pogotovo u kontrolnom netretiranom sjemenu. Tretiranje salicilnom kisjelinom je znatno poboljšalo neke osobine, kao što su procenat klijavosti, indeks klijavosti i rani porast sijanaca, u poređenju sa kontrolom. Nakon toga sijanci čička, koji su bili podvrgnuti predsjetvenim tretmanima, su uzgajani u hidroponskim uslovima u prisustvu kadmijuma (0-50 mg). Analiza lisnog tkiva sijanaca starih 30 dana otkrila je da je dejstvo katalaze (CAT) i gvajakol peroksidaze (GPX) pojačano povećanjem koncentracije kadmijuma. Međutim enzimatska aktivnost nije bila dovoljna za detoksikaciju reaktivnog kiseonika, tako da je primjena kadmijuma povećala stvaranje vodonik peroksida i znatno je uticala na gubitak integriteta ćelijske membrane. Smanjenje sadržaja ukupnog hlorofila i rastvorljivih proteina je uočen kod tretmana kadmijumom. Pa ipak, sijanci dobijeni od sjemena tretiranog salicilnom kiselinom pokazali su se bolje od ostalih predsjetvenih tretmana bilo u uslovima sa ili bez kadmijuma.

Ključne riječi: antioksidans, teški metal, ljekovita biljka, salicilna kiselina