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APPLICATION OF PROLINE AND MANNITOL ON *LEPIDIUM SATIVUM* L. UNDER ABIOTIC STRESS CONDITION (HEAVY METAL STRESS)

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Abstract

The impact of proline and mannitol to heavy metal stress on morphological, physiological and nutritional analysis were studied in *Lepidium sativum* L. Morphological parameters (shoot and root length) physiological parameters (carbohydrate, protein, proline and relative water content) nutritional analysis (sodium and potassium) were determined after 45 and 90 days. Exogenous application of proline and mannitol 50µg/l and 100µg/l each were standardized and applied to heavy metal stress ($CdSO_4$ 50µM and 100µM). Proline and mannitol enhanced the morphological, physiological parameters and nutritional factors in stressed plant; these played a major protagonist in cellular osmotic adjustment. Present study indicating that the proline and mannitol play essential role to heavy metal stress in *Lepidium sativum* L. Therefore, it found that the plants are able to cope with heavy metal stress when exogenous proline and mannitol is applied.

Key words: Proline, mannitol, heavy metal stress, protein, carbohydrate.

Introduction

Accumulation of heavy metal in plants induce the formation of complexes with oxygen, nitrogen and sulphur elements which shows several straight and indirect effects on growth of plant and alters numerous physiological functions. These complexes obstruct with mineral uptake, water relation, membrane functioning and protein metabolism (Drazic *et al*., 2004; Adhikari *et al*., 2006; Azevedo *et al*., 2005; Zhang *et al*., 2002; Shukla *et al*., 2003). Heavy metal stress is increasing day by day due to increase in level of mining operations, industrialization and public wastes (Clemens, 2006). Soil contaminated with the heavy metals above the allowable limit lead to declines in agricultural yields (Akinola and Ekiyoyo, 2006; Salt and Rauser, 1995). Heavy metals contaminate soil and depreciate the chemical and physiological composition of the plants which leads to many diseases in plants and animals. Among many heavy metals, cadmium is a major industrial pollutant particularly in areas associated with smelting of zinc and heavy road traffic (Das *et al*., 1997). Cadmium is not an essential nutrient and at high concentration inhibits plant growth (Aery and Rana, 2003). Due to cadmium toxicity it alters plant metabolism and cause growth inhibition, leaf chlorosis, and disturbance in photosynthesis are very common. The presence of cadmium in the soil decreases the growth of plants (Dewdy and Ham, 1997, Cataldo *et al*., 1983; Hasan *et al*., 2007). Proline accumulation in plants can serve as a biomarker of heavy metal stress. Proline increased greatly in leaves and less in stem under cadmium stress, more markedly under 100 µM Cd. Proline accumulation has also proposed as a mechanism of storage of excess nitrogen (Dinakar *et al*., 2007). It has been seen that when plants treated with toxic heavy metals they accumulate high concentrations of proline (Bassi and Sharma, 1993). Proline accumulation in plants also occurs in response to stresses such as salinity drought and low and high temperature (Naidu *et al*., 1991; Chu *et al*., 1976). It has been believed that proline accumulation is a symptom of injury which does not confer tolerance against metal or other stresses. Proline might protect plants from heavy metal toxicity (Kishor *et al*., 1995). In addition, mannitol is an important osmolytes, synthesized in numerous plant species (Abebe *et al*., 2003; Mitoi *et al*., 2009). It helps in storage of energy and carbon, osmoregulation and regulation of coenzymes. It plays a major functional role as an antioxidant due to its ability

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to search free radicals (Tandon *et al*., 2003). Application of mannitol increased plant biomass, photosynthetic pigments and antioxidant enzymes. It has also reported that mannitol plays a fundamental role in reducing osmotic and salinity induced stresses in many plants species (Tang *et al*., 2005).

Materials and Methods

Plant growth

The seeds propagated in seed trays comprising sand, soil, farmyard manure (FYM in ratio of 1:1:1) placed in a polyhouse with regulated temperatures ranging among 23 to 25°C, under a long-day photoperiod (16h light/8h dark). 10days old seedling shifted to different pots, which contain CdSO4 in different concentration 50 and 100µM/ kg soil. After shifting of 10 days to pots proline, mannitol 50µg/l and 100µg/l, each were standardized and applied to stress plants exogenously through foliar spray. Plants manured by adding Hoagland nutrient solution to each pot subsequently after every seven days. Plants parts (Leaves) sampled to determined morphological parameters (shoot and root length) physiological parameters (carbohydrate, protein, proline and relative water content) nutrients analysis (sodium and potassium) after 45 and 90 days.

Soot and root length

Shoot and root length of a *Lepidium sativum* L. measured by using scale.

Estimation of Carbohydrate

Total carbohydrate were determined in plant tissue method described by (Hedge *et al*., 1962). Weighed 100 mg of the sample. Hydrolyzed via keeping it in hot water bath aimed at 180 minutes through 5 mL of 2.5N HCl then cooled. Deactivated it through dense sodium carbonate until the bubbliness finishes. Centrifuged at 10,000 rpm for 5 minutes. Collected the supernatant and took 0.5 then 1ml aliquots for examination. Made up the volume toward 1ml in all the tubes comprising the sample tubes by addition of refined water. Then, added 4 ml of anthrone reagent. Heated aimed at eight minutes in a hot water bath. Cooled rapidly and read the green to dark green color at 630nm. Drawn a normal graph by scheming absorption of the standard on the X-axis versus absorbance proceeding the Y-axis from the graph calculated the quantity of carbohydrate existing in the sample tube.

Estimation of Protein Content

Protein estimated by method as described by (Lowry *et al*., 1951). Weighed 0.5gm of the sample then grind well through a pestle, mortar in 5-10 ml of the phosphate buffer. Centrifuged and cast-off the supernatant aimed at protein approximation. Pipette obtainable 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard hooked on a series of test tube. Pipette obtainable 0.2 ml of the sample extract in test tubes. A tube through 1mL of water aided as the blank. Added 5 ml of mixture C (alkaline copper solution) to each tube including the blank. Mixed well and permissible to stance for 10min. Then added 0.5 ml of reagent D (Folin-Ciocalteau Reagent) mixed well and kept at room temperature in the dark aimed at 30min. Blue color developed. Took the reading at 660nm. Drawn a standard graph using BSA and calculated the amount of protein in the sample.

Estimation of Proline Content

Proline measured by the method given by (Bates *et al.,* 1973). Extracted 0.5g of plant material by homogenizing in 10 ml of 3% aqueous sulphosalicylic acid. Filtered the homogenate through Whattman No. 2 filter paper. Took 2 ml of remainder in a test tube and added 2 ml of glacial acetic acid and 2 ml acid ninhydrin. Heated it in the boiling water bath for 1h. Terminated the reaction by retaining the tube in ice bath. Added 4 ml toluene to the reaction mixture and stirred well for 20-30secseparated the toluene layer and warmed to room temperature. Measured the red color intensity at 520nm. Ran a series of standards with pure proline in a similar way and prepared a standard curve.

Relative Water Content (RWC)

The fresh weight of top leaves from each treatment recorded. The leaves immersed in distilled water in beakers and left for 24 h. Thereafter, fully turgid leaves weighed again. The leaves dried in oven for 72 h at 70 ºC, until constant weight of leaves obtained. Relative water content (RWC) of leaves calculated according to (Wheatherley, 1950).

$$RWC = \frac{fresh\ mass - dry\ mass}{saturated\ mass - dry\ mass} \times 100$$

Determination of Sodium and Potassium

Potassium and sodium in the acid-digest of plant sample (leaf) was determined using flame photometer. Weighed 500 mg dried plant sample in 100 ml conical flask. Additional 10 ml of conc. HNO_3 placed funnel on the flask and kept for about 6-8 hrs or overnight at a covered place for pre-digestion. After pre-digestion when the solid sample was no more visible, additional 10 ml of conc. HNO_3 and 2-3 ml $HClO_4$. Kept on a hot plate in acid proof chamber having fume exhaust system, heated at about 100°C for first 1 hr, and then raised the temperature to 200°C. Continued digestion until the

contents became colorless and only white dense fumes appeared. Reduced the acid contents to about 2-3 ml by continuing heating at the same temperature. Filtered through Whattman No. 42 filter paper. Gave 3-4 washings of 10-15 ml portions of refined water then made the total volume 100 ml. Measured Na^+ and K^+ concentrations in the remainder by using Flame photometer. Recorded the flame photometer readings aimed at each of the operational standards of Na and K subsequently adjusting blank to zero. Drawn a standard curve by scheming the readings against Na and K readings.

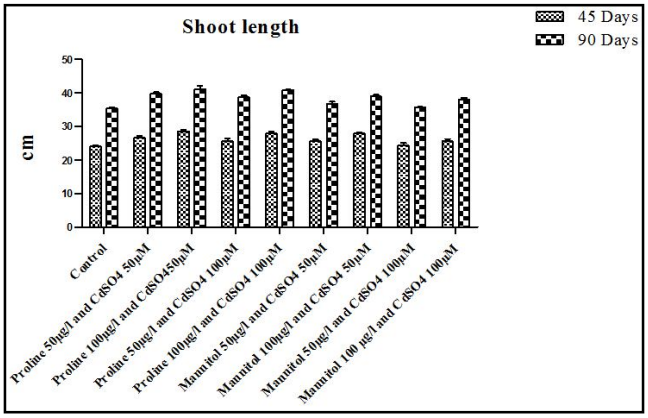

Fig. 1: Effect of proline and mannitol (50µg/l and 100µg/l) to heavy metal stress ($CdSO_4$ 50µM and 100µM) on shoot length.

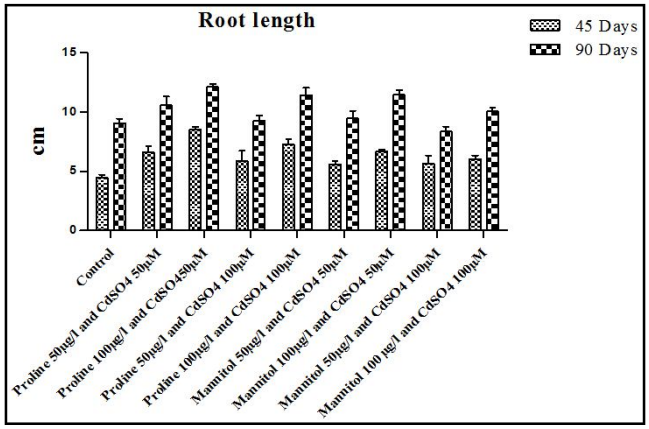

Fig. 2: Effect of proline and mannitol (50µg/l and 100µg/l) to heavy metal stress ($CdSO_4$ 50µM and 100µM) on root length.

Table 1: Effect of proline and mannitol to heavy metal stress on shoot length (cm) of *Lepidium sativum* L. Data are mean ± SD, of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonferroni multiple comparison post-test P<0.05*, P<0.01**, P<0.001*** significance level. Different lower case letters in a table indicate significant difference between control and treatments.

Treatments	45 Days	90 Days
Control	24.032±0.410a	35.361±0.202b,a
Proline 50µg/l and $CdSO_4$ 50µM	26.548±0.632a	39.66 3±0.502b,a
Proline 100µg/l and $CdSO_4$ 50µM	28.542 ±0.512a	41.162±0.891b,a
Proline 50µg/l and $CdSO_4$ 100µM	25.661±0.758a	38.664±0.573b,a
Proline 100µg/l and $CdSO_4$ 100µM	27.933±0.106a	40.833±0.124b,a
Mannitol 50µg/l and $CdSO_4$ 50µM	25.56 3±0.509a	36.813±0.513b,a
Mannitol 100µg/l and $CdSO_4$ 50µM	27.962±0.201a	38.965±0.452b,a
Mannitol 50µg/l and $CdSO_4$ 100µM	24.253±0.80a	35.660±0.152b,a
Mannitol 100 µg/l and $CdSO_4$ 100µM	25.613±0.651a	37.982±0.516b,a

Table 2: Effect of proline and mannitol to heavy metal stress on root length (cm) of *Lepidium sativum* L. Data are mean ± SD, of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonferroni multiple comparison post-test P<0.05*, P<0.01**, P<0.001*** significance level. Different lower case letters in a table indicate significant difference between control and treatments.

Treatments	45 Days	90 Days
Control	4.461±0.242a	9.071±0.361b,a
Proline 50µg/l and $CdSO_4$ 50µM	6.602±0.512a	10.562±0.722b,a
Proline 100µg/l and $CdSO_4$ 50µM	8.526±0.219a	12.121±0.233b,a
Proline 50µg/l and $CdSO_4$ 100µM	5.881±0.854a	9.261±0.433b,a
Proline 100µg/l and $CdSO_4$ 100µM	7.262±0.442a	11.412±0.608b,a
Mannitol 50µg/l and $CdSO_4$ 50µM	5.601±0.271a	9.451±0.642b,a
Mannitol 100µg/l and $CdSO_4$ 50µM	6.704±0.125a	11.422±0.422b,a
Mannitol 50µg/l and $CdSO_4$ 100µM	5.637±0.704a	8.342±0.427b,a
Mannitol 100 µg/l and $CdSO_4$ 100µM	6.033±0.302a	10.055±0.314b,a

Results

Shoot and root length

Shoot and root length of *Lepidium sativum* L. increased significantly upon exposure of osmolyte that are proline and mannitol 50µg/l, 100µg/l along with heavy metal stress ($CdSO_4$ 50µM, 100µM) in comparison to their respective control at 45 and 90 days shown in table 1, 2 and Fig. 1, 2. Shoot and Root length increase maximum in case of proline as compared to mannitol.

Relative water content

The effect of proline and mannitol 50µg/l, 100µg/l along with heavy metal stress ($CdSO_4$ 50µM, 100µM) on relative water content of *Lepidium sativum* L. explained in table 3 and Fig. 3. Relative water content increased in concentration dependent manner. It increase maximum in context of proline as compared to the mannitol.

Carbohydrate

The effects of osmolyte proline and mannitol

along with heavy metal stress treatments on carbohydrate content of *Lepidium sativum* L. shown in table 4 and Fig. 4. The carbohydrate content enhanced in concentration dependent manner. While proline and

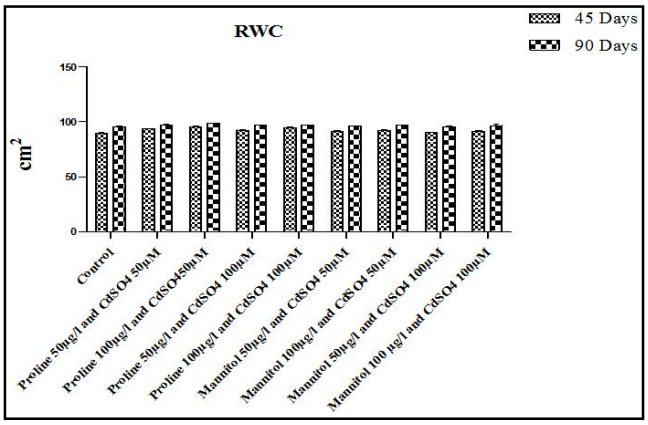

Fig. 3: Effect of proline and mannitol (50µg/l and 100µg/l) to heavy metal stress ($CdSO_4$ 50µM and 100µM) on Relative water content.

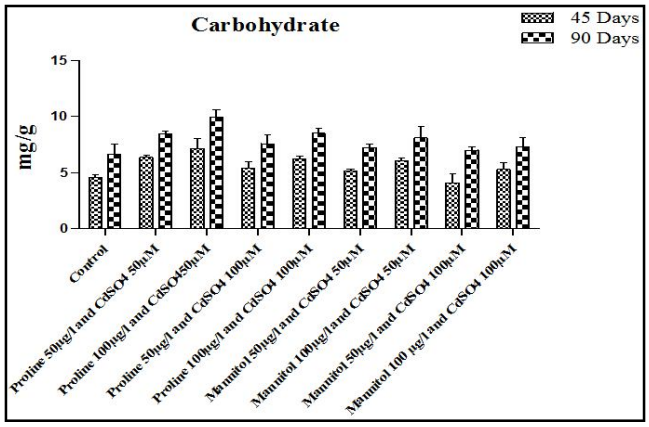

Fig. 4: Effect of proline and mannitol (50µg/l and 100µg/l) to heavy metal stress ($CdSO_4$ 50µM and 100µM) on carbohydrate content.

Table 3: Effect of proline and mannitol to heavy metal stress on relative water content (cm^2) of *Lepidium sativum* L. Data are mean ± SD, of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonferroni multiple comparison post-test P<0.05*, P<0.01**, P<0.001*** significance level. Different lower case letters in a table indicate significant difference between control and treatments.

Treatments	45 Days	90 Days
Control	89.831±0.973a	95.607±0.362b,a
Proline 50µg/l and $CdSO_4$ 50µM	93.855±0.128a	97.081±0.347b,a
Proline 100µg/l and $CdSO_4$ 50µM	95.421±0.923a	98.772±0.227b,a
Proline 50µg/l and $CdSO_4$ 100µM	92.408±0.778a	96.822±0.544b,a
Proline 100µg/l and $CdSO_4$ 100µM	94.781±0.614a	97.134±0.219b,a
Mannitol 50µg/l and $CdSO_4$ 50µM	91.561±0.378a	96.152±0.217b,a
Mannitol 100µg/l and $CdSO_4$ 50µM	92.218±0.561a	97.121±0.191b,a
Mannitol 50µg/l and $CdSO_4$ 100µM	90.201±0.451a	95.758±0.281b,a
Mannitol 100 µg/l and $CdSO_4$ 100µM	91.593±0.402a	96.574±0.931b,a

Table 4: Effect of proline and mannitol to heavy metal stress on carbohydrate content (mg/g) of *Lepidium sativum* L. Data are mean ± SD, of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonferroni multiple comparison post-test P<0.05*, P<0.01**, P<0.001*** significance level. Different lower case letters in a table indicate significant difference between control and treatments.

Treatments	45 Days	90 Days
Control	4.521±0.298a	6.647±0.889b,a
Proline 50µg/l and $CdSO_4$ 50µM	6.329±0.164a	8.458±0.198b,a
Proline 100µg/l and $CdSO_4$ 50µM	7.132±0.881a	9.916±0.678b,a
Proline 50µg/l and $CdSO_4$ 100µM	5.370±0.571a	7.561±0.787b,a
Proline 100µg/l and $CdSO_4$ 100µM	6.174±0.271a	8.484±0.478b,a
Mannitol 50µg/l and $CdSO_4$ 50µM	5.139±0.155a	7.197±0.329b,a
Mannitol 100µg/l and $CdSO_4$ 50µM	6.048±0.188a	8.094±0.978b,a
Mannitol 50µg/l and $CdSO_4$ 100µM	4.057±0.778a	6.992±0.261b,a
Mannitol 100 µg/l and $CdSO_4$ 100µM	5.259±0.589a	7.257±0.878b,a

mannitol 50µg/l, 100µg/l applied with $CdSO_4$ 50µM, 100µM then carbohydrate content is significantly increased as compare to their respective control at 45 and 90 days.

Protein

The effects of proline and mannitol along with heavy metal stress treatments on protein content of *Lepidium sativum* L. shown in table 5 Fig. 5. The protein content is enhanced in concentration dependent manner. While proline and mannitol 50µg/l, 100µg/l applied with $CdSO_4$ 50µM, 100µM then protein content is significantly increased as compare to their control at 45 and 90 days.

Proline

The effects of osmolyte proline and mannitol to heavy metal stress treatments on proline content of *Lepidium sativum* L. shown in table 6 Fig. 6. The proline content is enhanced in concentration dependent manner. While proline and mannitol 50µg/l, 100µg/l applied with $CdSO_4$ 50µM, 100µM then proline content is significantly increased as compare to their respective control at 45 and 90 days. It increase maximum in case of proline as compared to mannitol.

Sodium

The effects of proline and mannitol to heavy metal stress treatments on sodium content of *Lepidium sativum* L. shown in table 7 Fig. 7. The sodium content enhanced in concentration dependent manner. While proline and mannitol 50µg/l, 100µg/l applied with ($CdSO_4$ 50µM,

100µM) then sodium content is significantly increased as compare to their control at 45 and 90 days.

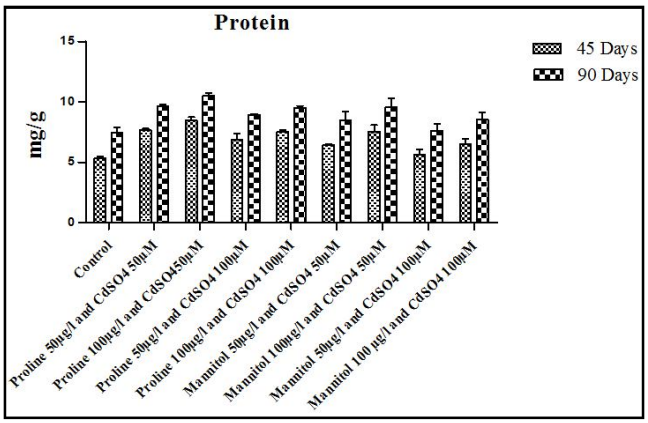

Fig. 5: Effect of proline and mannitol (50µg/l and 100µg/l) to heavy metal stress ($CdSO_4$ 50µM and 100µM) on protein content.

Potassium

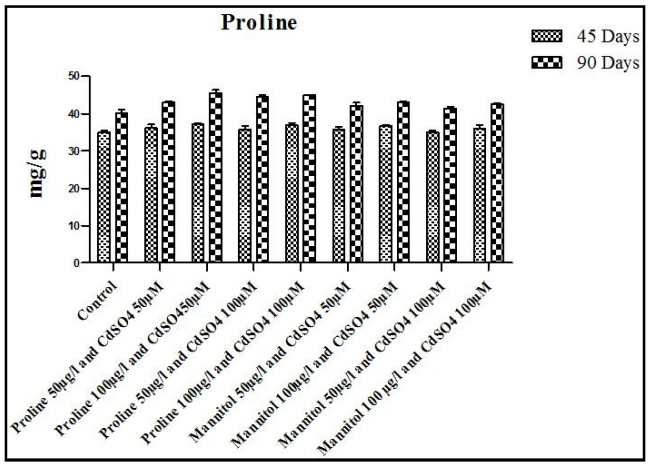

Fig. 6: Effect of proline and mannitol (50µg/l and 100µg/l) to heavy metal stress ($CdSO_4$ 50µM and 100µM) on proline content.

Table 5: Effect of proline and mannitol to heavy metal stress on protein content (mg/g) of *Lepidium sativum* L. Data are mean ± SD, of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonferroni multiple comparison post-test P<0.05*, P<0.01**, P<0.001*** significance level. Different lower case letters in a table indicate significant difference between control and treatments.

Treatments	45 Days	90 Days
Control	5.309±0.155a	7.431±0.478b,a
Proline 50µg/l and $CdSO_4$ 50µM	7.651±0.137a	9.664±0.112b,a
Proline 100µg/l and $CdSO_4$ 50µM	8.488±0.254a	10.491±0.244b,a
Proline 50µg/l and $CdSO_4$ 100µM	6.881±0.487a	8.899±0.101b,a
Proline 100µg/l and $CdSO_4$ 100µM	7.501±0.161a	9.521±0.141b,a
Mannitol 50µg/l and $CdSO_4$ 50µM	6.414±0.101a	8.454±0.745b,a
Mannitol 100µg/l and $CdSO_4$ 50µM	7.531±0.545a	9.584±0.678b,a
Mannitol 50µg/l and $CdSO_4$ 100µM	5.591±0.498a	7.612±0.554b,a
Mannitol 100 µg/l and $CdSO_4$ 100µM	6.454±0.521a	8.551±0.592b,a

Table 6: Effect of proline and mannitol to heavy metal stress on proline content (mg/g) of *Lepidium sativum* L. Data are mean ± SD, of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonferroni multiple comparison post-test P<0.05*, P<0.01**, P<0.001*** significance level. Different lower case letters in a table indicate significant difference between control and treatments.

Treatments	45 Days	90 Days
Control	34.954±0.427a	40.262±0.785b,a
Proline 50µg/l and $CdSO_4$ 50µM	36.137±0.945a	43.162±0.102b,a
Proline 100µg/l and $CdSO_4$ 50µM	37.208±0.178a	45.537±0.865b,a
Proline 50µg/l and $CdSO_4$ 100µM	35.751±0.845a	44.411±0.665b,a
Proline 100µg/l and $CdSO_4$ 100µM	36.861±0.645a	44.934±0.114b,a
Mannitol 50µg/l and $CdSO_4$ 50µM	35.750±0.731a	42.154±0.789b,a
Mannitol 100µg/l and $CdSO_4$ 50µM	36.691±0.223a	43.157±0.117b,a
Mannitol 50µg/l and $CdSO_4$ 100µM	35.050±0.445aa	41.451±0.342b,a
Mannitol 100 µg/l and $CdSO_4$ 100µM	35.962±0.956	42.611±0.165b,a

The effects of osmolyte proline and mannitol to heavy metal stress treatments on potassium content of *Lepidium sativum* L. shown in table 8 Fig. 8. The sodium content enhanced in concentration dependent manner. While proline and mannitol 50µg/l, 100µg/l applied with $CdSO_4$ 50µM, 100µM then potassium content is significantly increased as compared to their respective control at 45 and 90 days. It increase maximum in context to proline as compared to the mannitol.

Discussion

In the present study, the results revealed that morphology (shoot and root length) physiology (carbohydrate, protein, proline, relative water content) nutritional (sodium and potassium) of *Lepidium sativum* L. showed an enhancement under heavy metal stress ($CdSO_4$) condition along with exogenous application of proline and mannitol in concentration dependent manner. An increase in carbohydrate content also observed under cadmium stress with the exogenous application of proline and mannitol is applied. The degree of enhancement of carbohydrates was maximum in proline and minimum in mannitol. Protein synthesis also affected by cadmium treatments. The reduction for protein could be due to decrease in protein synthesis or an increase in the rate of protein degradation (Balestrasse *et al*., 2003). It is evident from the results that with an increase in the concentration of heavy metal stress protein content decreased but enhanced due to the

exogenous application of proline and mannitol. It is evident from the results that with an increase in the concentration

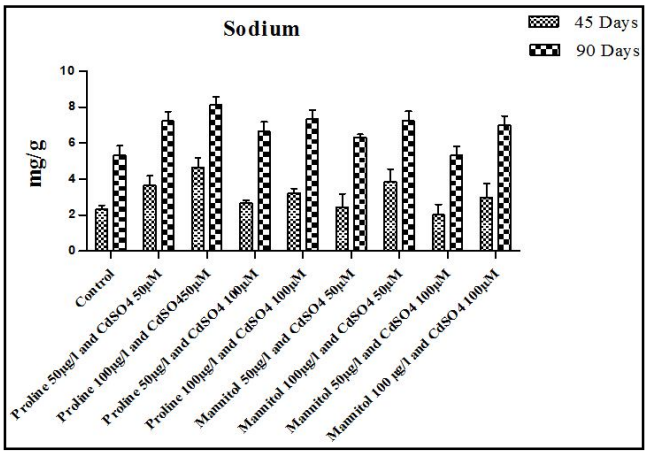

Fig. 7: Effect of proline and mannitol (50µg/l and 100µg/l) to heavy metal stress ($CdSO_4$ 50µM and 100µM) on sodium content.

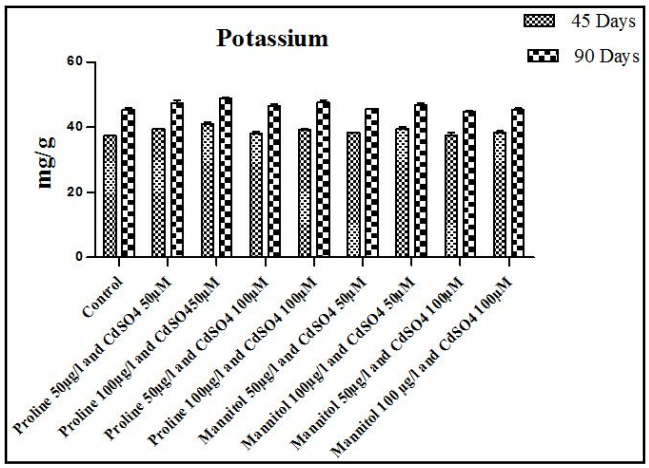

Fig. 8: Effect of proline and mannitol (50µg/l and 100µg/l) to heavy metal stress ($CdSO_4$ 50µM and 100µM) on potassium content.

Table 7: Effect of proline and mannitol to heavy metal stress on sodium content (mg/g) of *Lepidium sativum* L. Data are mean ± SD, of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonferroni multiple comparison post-test P<0.05*, P<0.01**, P<0.001*** significance level. Different lower case letters in a table indicate significant difference between control and treatments.

Treatments	45 Days	90 Days
Control	2.325±0.225a	5.339±0.522b,a
Proline 50µg/l and $CdSO_4$ 50µM	3.667±0.523a	7.234±0.504b,a
Proline 100µg/l and $CdSO_4$ 50µM	4.661±0.521a	8.125±0.452b,a
Proline 50µg/l and $CdSO_4$ 100µM	2.66±0.152a	6.661±0.511b,a
Proline 100µg/l and $CdSO_4$ 100µM	3.231±0.241a	7.361±0.478b,a
Mannitol 50µg/l and $CdSO_4$ 50µM	2.444±0.732a	6.334±0.155b,a
Mannitol 100µg/l and $CdSO_4$ 50µM	3.851±0.714a	7.248±0.522b,a
Mannitol 50µg/l and $CdSO_4$ 100µM	2.023±0.574a	5.338±0.491b,a
Mannitol 100 µg/l and $CdSO_4$ 100µM	2.986±0.784a	6.984±0.524b,a

Table 8: Effect of proline and mannitol to heavy metal stress on potassium content (mg/g) of *Lepidium sativum* L. Data are mean ± SD, of three replicates (n=3) were analyzed using graph pad prism 5.2 by Two way Anova followed by Bonferroni multiple comparison post-test P<0.05*, P<0.01**, P<0.001*** significance level. Different lower case letters in a table indicate significant difference between control and treatments.

Treatments	45 Days	90 Days
Control	37.314±0.107a	45.36±0.567b,a
Proline 50µg/l and $CdSO_4$ 50µM	39.334±0.229a	47.308±0.921b,a
Proline 100µg/l and $CdSO_4$ 50µM	40.905±0.659a	48.664±0.531b,a
Proline 50µg/l and $CdSO_4$ 100µM	38.014±0.604a	46.612±0.504b,a
Proline 100µg/l and $CdSO_4$ 100µM	39.262±0.214a	47.541±0.761b,a
Mannitol 50µg/l and $CdSO_4$ 50µM	38.321±0.114a	45.614±0.124b,a
Mannitol 100µg/l and $CdSO_4$ 50µM	39.424±0.634a	46.657±0.651b,a
Mannitol 50µg/l and $CdSO_4$ 100µM	37.454±0.721a	44.761±0.214b,a
Mannitol 100 µg/l and $CdSO_4$ 100µM	38.259±0.505a	45.312±0.451b,a

of heavy metal stress along with exogenously applied proline and mannitol the proline content decreased. There was a progressive decrease in the proline content in 100µM $CdSO_4$ and 100µg/l proline as compared to mannitol. The degree of decrease in proline content was minimum in 100µM $CdSO_4$ and 100µg/l mannitol and maximum in 100µM $CdSO_4$ and 100µg/l proline. It has suggested that proline accumulation in plants under $CdSO_4$ stress is due to the decrease of the plant water potential and the functional signiûcance of this accumulation could related to water balance (Schat and Vooijs, 1997). Relative water content (RWC) is the measure of health and sturdiness in a plant and in stress growing plants; it is lower (Bhardwaj and Yadav, 2012). Relative water content enhanced with time in *Lepidium sativum* L. growing in heavy metal stress conditions due to the application of exogenously applied proline and mannitol. In present study the heavy metal stress enhanced the sodium content of *Lepidium sativum* L. Similar results has also been seen in *Brassica juncea* where the leaf content of Na^+ increased gradually on increase in $CdSO_4$ level (Lakra *et al*., 2006). It is evident from the results that with an increase in the concentration of heavy metal stress Na^+ content are increased and balanced by the application of proline and mannitol. When the osmolyte is applied, they decreased the sodium content as compared to their respective control. There was a progressive decrease in the sodium content in proline is more as compared to

mannitol. The degree of decrease in sodium content was maximum in proline and minimum in mannitol. Potassium is other essential macronutrients, taken up by the roots and generally transported to the shoot through the xylem and this transport seems controlled by the shoot growth (Lidon and Henriques, 1991). Potassium plays an important role in balancing membrane potential and turgor, activating enzymes, regulating osmotic pressure, stoma movement and membrane polarization (Maathuisand Sanders, 1996; Kaya *et al.,* 2007). It is evident from the results that with an increase in the concentration of heavy metal stress K^+ contents decreased. When the osmolyte proline and mannitol is applied, they enhanced the K^+ content in *Lepidium sativum* L. There was a progressive enhancement in the K+ content in stressed plants due the application of proline and mannitol as compared to their respective control. The degree of enhancement maximum in case of proline as compared to mannitol.The aim of present study were determined the importance of exogenous application of osmolytes proline and mannitol on the alleviation of cadmium sulphate ($CdSO_4$) induced toxicity effects on morphology, physiology and nutritional responses of *Lepidium sativum* L.

Conclusion

The present study suggested that the *Lepidium sativum* L. maintained physio-biochemical responses during heavy metal stress, which may be due to efficient osmotic regulation maintained by exogenous application of osmolytes proline and mannitol. Generally, accumulation of osmolytes increases under stress condition, which not only assistances in maintaining cell turgor is also involved in quenching free radicals. Exogenous application of proline and mannitol along with heavy metal stress then the shoot length, root length, relative water content, carbohydrate, protein, proline, sodium and potassium enhanced as an significantly compare to their respective control.

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