International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 12, No. 5, p. 86-98, 2018

INNSPUB

RESEARCH PAPER OPEN ACCESS

Interactive effects of cadmium stress and proline on physiological and biochemical parameters of faba bean plant

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Key words: Growth, metal stress, cadimium chloride, *Vicia faba,* proline.

http://dx.doi.org/10.12692/ijb/12.5.86-98 Article published on May 24, 2018

Abstract

Heavy metal stress is one of the major abiotic stresses that cause environmental pollution in recent decades. Under stress, Proline accumulated in many plants and acts as a signaling molecule and trigger specific gene expression, which can be essential for plant recovery from stress .Therefore, the present experiment was aimed to study the effect of proline on the performance of faba bean under cadmium stress condition. The effect of exogenous application of proline with different concentrations (0, 25 or 50mg / l) on faba bean (*Vicia faba*) plant grown under cadimium levels (0 or 150µM/l).Under Metal stress condition, all parameters [plant height and root length, root and shoot fresh weight, total soluble carbohydrates (TSC), chlorophyll (Chl) *a* and *b*, and total chlorophyll of faba beans were strongly depressed, except malondialdehyde (MDA) and proline contents. From these results, proline treatment alleviated the adverse effects of metal stress through increased the photosynthetic pigments, total carbohydrates, plant height and fresh weights of shoot, and seed yield as well as, total soluble sugars, compared with those of the corresponding cadimium levels, while decreased lipid peroxidation product as malondialdehyde (MDA) and the content of proline. These results indicate that application of proline was effective, and helped the plant to restore the altered physiological process induced by cadmium stress.

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Introduction

Bean seeds (*Vicia faba*) are also a rich source of mineral salts, phosphorus, magnesium and iron as well as group B vitamins. Cultivated green-podded string beans are distinguished by a higher vitamin content and therefore have a higher biological value than yellow podded varieties (*Crépon et al., 2010*). Faba bean is cultivated in sustainable cropping systems due to its ability of providing nitrogen by biological fixation and consequent reduction of chemical inputs consumption in plant production (Ghanbari *et al.,* 2010; Stoltz and Nadeau, 2014).

Of the implications of human-induced disturbance of natural biogeochemical cycles, accentuated accumulation of heavy metals is a problem of paramount importance for ecological, nutritional, and environmental reasons (Nagajyoti *et al.,* 2010 ; Ali *et al.*,2013).

Cadmium (Cd) is a toxic heavy metal. It has an important impact on agriculture, as the excessive consumption of Cd from contaminated food crops can lead to toxicity in humans(Wagner ,1993).Due to its high mobility and water solubility, Cd enters readily the roots where it is mainly retained (Lux *et al.,* 2011). The cellular toxicity can result from various direct as well as indirect effects of Cd. This heavy metal targets and damages of cellular activities and processes such as photosyn- thesis, carbohydrate and nitrate metabolism, water balance, DNA and lipid matrix, resulting in growth inhibition or in plant senescence or even in plant death (Dal Corso *et al.,* 2008). Plants in natural environments are subject to a variety of stress, which have negative effects on their growth. (Nagajyoti *et al.,* 2010). Plants respond to drought stress at the physiological, cellular and molecular levels, but the extent of damage depends on the age and stage of plant growth. To fight against abiotic stress, plant has developed mechanisms to minimize the potential heavy metal damages. Within the cell is detoxified preferentially by binding to S-containing ligands such as metallothioneins (MTs),glutathione (GSH) and phytochelatins (PCs). Further the ligand–Cd complexes are most likely removed by sequestration from potentially sensitive organelles and structures (Cobbett and Goldsbrough, 2002; Clemens, 2006). Under stress, proline accumulated in many plants and acts as a signaling molecule and trigger specific gene expression, which can be essential for plant recovery from stress (Szabados and Savouré, 2009). However, proline is a universal osmoprotectant and antioxidant, as well as a source of energy, reducing equivalent, N and carbon and is a proteinogenic amino acid (Kuznetsov and Shevyakova, 1999; Matysik *et al*., 2002; Szabados and Savouré, 2009; Ali *et al*., 2011). Accumilation are not yet completely understood (Szabados and Savouré, 2009).Moreover, proline accumulation in plants could be only useful as a possible drought injury sensor instead of its role in stress tolerance mechanism (Jahari *et al.,* 2010). In addition, Vendruscolo *et al.,* (2007) reported that proline is involved in tolerance mechanisms against oxidative stress and this is the main strategy of plants to avoid detrimental effects of stress. Proline plays an important role as a sink for energy to regulate redox potentials (Simiroff and Cumbes, 1989), and protects plants from free radical that induced damage by quenching of singlet oxygen (Matysik *et al.,* 2002).Therefore, the present study was carried out to examine the effect of proline treat (0, 25 and 50 mg/l) on some physiological and biochemical attributes of *Vicia faba* bean plants under Cd treatment (150 µM/l).

Materials and methods

Conditions of Plant Cultivation

The seeds of *Vicia faba* are hydrated for twenty-four hours in water at room temperature. Seeds washed with tap water for 15 minutes then sterilized by 2 % v/v sodium hypochlorite for 15 minutes and then washed extensively with sterilized distilled water. Petri dishes used with filter paper when working with seedlings, cultivation conducted at 22° C. The seedlings are then transferred to a hydroponic culture device in a phytotron allowing optimal growth of the plants. The roots are regularly oxygenated by a bubbling system. After two weeks, the plants (5th-6th leaf stage) are placed in individual vats to undergo various treatments. The pots were divided into six groups. The first group (Cd0P0) to serve as control, the second group cultivated with hydroponic solution with 25mg/l proline (Cd0P1).the third group cultivated with hydroponic solution with 50 mg/l proline (Cd0 P2) , the group number 4 cultivated with hydroponic solution with 150 µM of Cd (Cd150PO) , the group number 5 cultivated with hydroponic solution with 150 µM of Cd and 25 mg/l of proline (Cd150 P1) and the group number 6 cultivated with hydroponic solution with 150 µM of Cd and 50 mg/l of proline (Cd150 P2). At the end of the experiment, sampling is done on both leaves and roots immediately frozen.

Measurements

Three weeks after the application of Cd and proline (about 30 days after germination), three replicates were taken from each treatment, and the following parameters were measured:

Determination of growth parameters

The plants were washed, fresh weight (FW) determined using sensitive electrical balance and roots and shoots separated. Root and shoot lengths were measured.

Determination of Photosynthetic pigments

The photosynthetic pigments (chlorophyll a and chlorophyll b were determined spectrophotometrically according to Metzner *et al.,* (1965).

Determination of membrane characteristics

Lipid peroxidation level was measured as the content of malonyldialdehyde (MDA) using the thiobarbioturic method (Zhao *et al.,* 1994), and expressed as nmol of MDA formed using an extinction coefficient of 155 mM-1 cm-1 .

Determination of soluble sugars

Soluble sugar was extracted from dried leaf tissue with 80% ethanol. The soluble sugars were determined by the anthrone sulfuric acid method described by Scott and Melvin, (1956).

Determination of free proline content

The free proline was estimated according to Bates *et al.,* (1973). The color intensity was determined spectrophotometrically at 520 nm on a spectrophotometer.

Statistical analysis

Results were expressed as mean ± SD of three separate experiments. For each experiment, statistical analysis was performed using one-way ANOVA and the multiple comparison method of Tukey, with the aid of R 3.4.3 computer program.

Results

Data in Fig.1 clearly show that, growth parameters (shoot and root length, shoot, root and plant fresh weights) were reduced gradually and significantly with increasing heavy metal levels in faba bean plant.

Proline treatments caused stimulatory effects on such parameters under both Cd and non-Cd (control) conditions. Proline amino acid treatments alleviated the inhibitory effect of heavy metal stress on the above mentioned parameters.

Photosynthetic pigments

Cd stress reduced gradually, chlorophyll *a*, chlorophyll *b* and total pigment contents of faba bean leaves Fig.2. The chlorophyll content reflects the photosynthesis rate of plant, which strongly influenced by environmental factors.

Proline

Fig. 3 illustrated that proline accumulation in faba bean plants increased significantly and gradually with increasing Cd concentration relative to control (Cd0P0) treatment.

However, application of proline decreased the concentration of proline content in faba bean plants.

Malon dialdehyde (MDA)

Fig.4 showed that Cd treat caused a significant increase in amount of Malon dialdehyde (MDA), compared with the control plants.

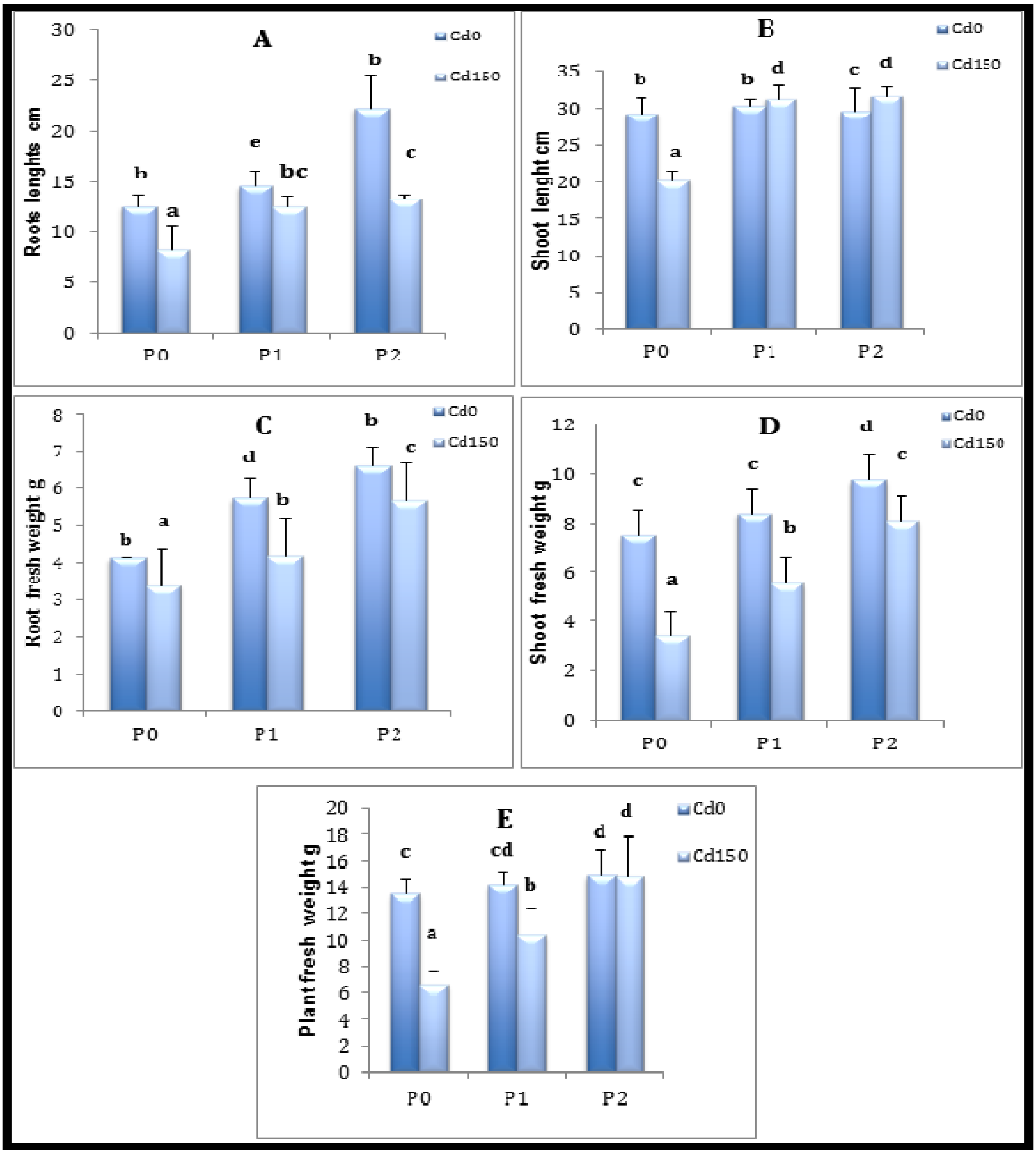

Fig.1. Effect of proline on root length (A), shoot lenght (B), root fresh weight (C), shoot fresh weight (D) and Total plant fresh weight (E) of faba bean plants. Bars with the same letter do not differ at the P<0.05 level of significance (Multiple Comparisons of Means: Tukey Contrasts).

However, treatment of the stressed plants with proline caused a significant decrease in the MDA compared with those of the reference controls.

Total soluble carbohydrates

Fig.5 shows that Cd caused significant decreases in total carbohydrate contents of faba bean shoots compared with control. Application of proline with differents concentrations increased significantly total carbohydrates compared with control. These increases were correlated positively with increasing its concentration.

Discussion

In the present experiment, plant growth parameters, root length, shoot length, root fresh weight and shoot fresh weight decreased with increasing metal stress. Similar data were recorded by (Wang *et al.*, 2016).

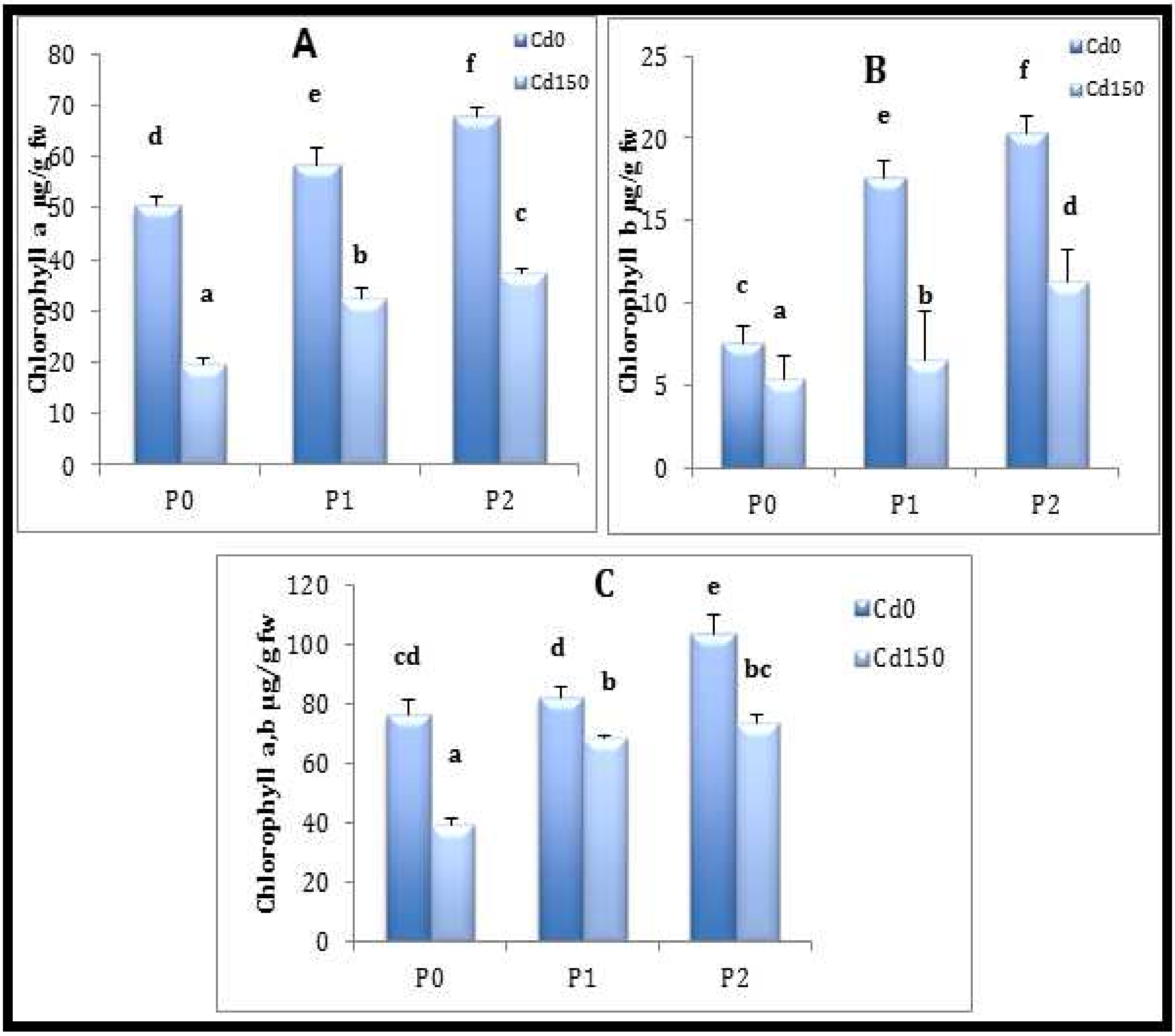

Fig. 2. Effect of proline on the content of Chl a (A) , Chl b (B) and total Chl a,b (C).in faba bean plants.

A decrease in plant growth might be due to inhibition of cell en largement and cell division, and reduction of various plant growth metabolisms (Yordanov *et al.,* 2003; Farooq *et al.,* 2008). The lower susceptibility of roots might be explained by accumulation of the metal in inactive form or else by mobility of Cd within the plant, facilitating its translocation to aerial parts (Barceló *et al.,* 1988). In contrast to our results, Titov *et al.,* (1996) reported higher sensitivity of roots in heavy metal treated wheat and barley seedlings. In maize cultivars, increased Cd in nutrient solution significantly reduced the dry weight of both leaves and roots (Ekmekçi *et al*., 2008). Apart from the impact of Cd in the growth medium and concomitant accumulation in roots, it seems that root growth in poplars also depends on the genotype. Pilipović *et al.,* (2005) grew poplar plants hydroponically in the presence of 0, 10-5 and 10-7 M Cd. The growth responses of roots and shoots exhibited differences between four poplar clones, and in some cases biomass production was stimulated. Lower biomass production (especially in leaves) and shorter roots are symptoms of Cd toxic- ity described previously in tomato plants (*Chaffei et al*., 2004).Application of proline with 50mg was found to be more effective in comparaison to application of proline with 25mg concentration. This result strengthens the findings of Ali *et al.,* 2007, who reported that exogenous application of Pro improved plant growth of maize under water stress condition.The effectiveness of proline application on plants depends on the type of species, plant developmental stage, time of application and concentration.

Moreover, under adverse environmental conditions, the effect of proline application is species specific (Ashraf and Foolad, 2007).

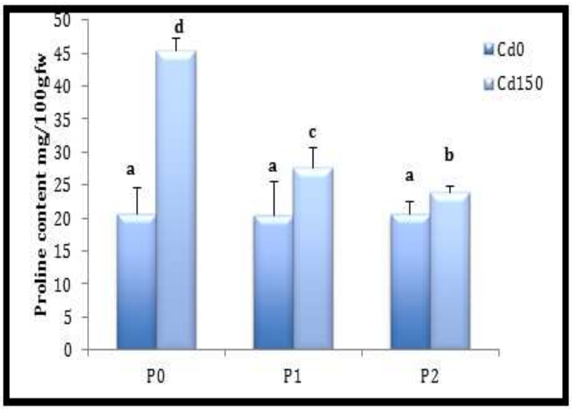

Fig.3. Interactive effects of different concentrations of proline (P) and different levels of cadimium (Cd) on free proline content of faba bean plant.

The results showed that chlorophyll a,b and total pigments were negatively affected by application of Cd stress. These results are similar with those findings by, Heuer (2005) found that chlorophyll content increased in tomato under low levels of salinity. The decrease in chlorophyll content under stress conditions was reported by Vassilev *et al.,*(2005) ; Kusvuran, (2010) and Nazarbeygi *et al.,*(2011) and might have been due to inhibition of enzymes associated with chlorophyll biosynthesis (Dong *et al.,* 2014) inhibition of uptake and transportation ofother metal elements such as Mn, Zn, and Fe byantagonistic effects (John *et al.,* 2009). Similar decrease in chlorophyll content under heavy metal stress wasreported in *Atriplex halimus* (Brahim and Mohamed,2011) and mangrove plant seedlings (Zhang *et al.,*2007). The results obtained in this study are in agreement with those of Rahimi *et al.,*(2012) on Azooz *et al.,* (2013) on *Vicia. faba*. The decrease in chlorophyll content in stressed faba bean plants concomitant with the increase in proline content is consistent with the suggestion that nitrogen might be redirected to the synthesis of proline instead of chlorophyll. In addition, Bajguz (2011) ascribed the suppressed pigment content in heavy metal stressed plants to increased activity of chlorophyllase or disruption of the fine structure of the chloroplast, as well as instability of the chloroplast membrane and pigment protein complex. In this regard, Sevengor *et al.,*(2011) and Emamverdian *et al.,* (2015) attributed the reduction in leaf chlorophyll content under heavy metal and salt stress to the destruction of chlorophyll pigments and the instability of the pigment protein complex. Ali *et al.,*(2007) explained the beneficial effect of proline applied was due to its promotive effects on photosynthetic capacity by overcoming stomata limitations, enhancing biosynthesis of photosynthetic pigments, or protecting photosynthetic pigments from Cd stress-induced degradation.

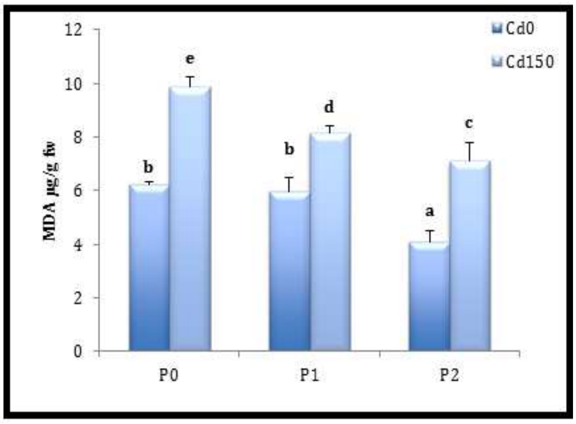

Fig. 4. Interactive effects of different concentrations of proline (P) and different levels of cadimium (Cd) on MDA content of faba bean plant.

The result shows that proline accumulation increased as Cd levels increased with the highest amount found in untreated plants. Early studies showed that growing plants under heavy metal stress increased proline content (Aly and Mohamed, 2012; Karimi *et al.*, 2012). Accumulation of free proline in response to heavy metal exposure seems widespread among plants (Thounaojam *et al.*, 2012). Various earlier studies reported that proline contents significantly increased in many species including common bean (Khadri *et al.,* 2006), corn (Yoon *et al.,* 2005) and soybean (Chon *et al.,* 2003) under stress conditions. The exposure to heavy metals, especially Cd is known to disturb the plant water balance. It is well known that proline prevents membrane damage and had a protective role in lipid proxidation induced by metals (Thounaojam *et al.*, 2012). Proline accumulation in plants under Cd stress is induced by a heavy metal imposed decrease of the plant water potential, and the functional significance of this accumulation would lie in its contribution to water balance maintenance; proline-mediated alleviation of water deficit stress could substantially contribute to Cd and Pb tolerance (Zengin and Munzuroglu, 2005).subjected to Pb and Cd stress (John *et al.,* 2009) *Solanum nigrum L.* exposed to Cu stress (Fidalgo *et al.,* 2002), wheat subjected to Cd and lemongrass (Cymbopogon flexuosus Stapf) subjected to Hg and Cd stress (Handique *et al.,* 2009).

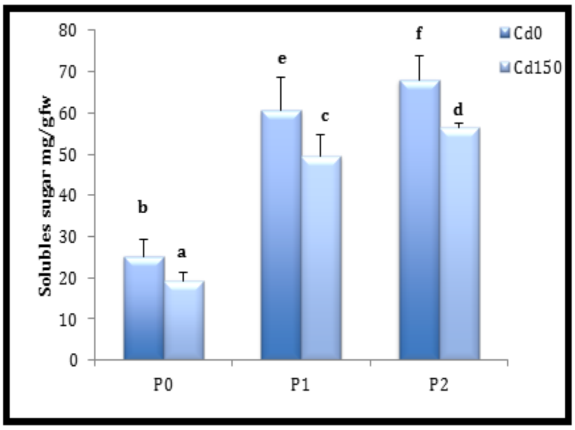

Fig.5. Interactive effects of different concentrations of proline (P) and different levels of cadimium (Cd) on total soluble carbohydrate of faba bean plant.

In hybrid poplar (*Populus trichocarpa* × deltoides), it was shown that proline accumulation in roots was almost 2-fold higher than that of leaves when Cd was applied in strong doses In view of some earlier reports who suggested that exogenously proline applied might have enhanced endogenous proline accumulation under water stress conditions which not only protects enzymes, 3D structures of proteins and organelle membranes, but it also supplies energy for growth and survival there by helping the plant to tolerate stress (Ashraf and Foolad, 2007; Hoque *et al.*,2007). The accumulation of proline concomitant with increasing stress in faba bean plants was in agreement with the results obtained by John *et al.,* (2008) and Rahimi *et al.,* (2012). In addition, proline accumulation was reported to serve as a nitrogen storage compound and thus protectcellular structure (Rahimi *et al.*,2012).

The increase in MDA content in the plants grown in heavy metal stress indicates that Cd stress may be responsible for increasing lipid peroxidation leading to cell damage. This result is in accordance with previous findings of other workers (Sairam *et al.*,2000; Moussa and Abdel-Aziz, 2008). However**,** the rapid induction of proline in roots and forming Pro-metal complexes may offer a better and effective way of nullifying toxicity of metals rather than allowing them to reach above-ground parts. Theriappan *etal.*,(2011) experimenting with cauliflower seedlings (Brassica oleracea var. botrytis) and three heavy metals (Cd, Hg, and Zn) noticed the concentration-dependent accumulation of proline in which intensification of toxicity (up to 1000 µM) almost doubled the production of proline. Additionally, they showed that Hg was the strongest initiator of proline. In another study using sal seedling (*Shorea robusta*), it was determined that Cd, Pb, and as were, respectively, stronger evokers of proline (Pant *et al.,* 2011) .

The interaction effect of heavy metal combined with proline amino acid was significant in total carbohydrates. Total carbohydrate in faba bean leaves was negatively affected by Cd stress. The negative effect of heavy metals on carbon metabolismis a result of their possible interaction with the reactive centre of ribulose bisphosphate carboxylase (John *et al.*, 2008).Soluble sugar is an important constituent manufactured during photosynthesis and break down during respiration by plants. All metals have decreased the content with increasing concentration as reported in agricultural crops (Rascio and Navari-Izzo, 2011). Such inhibition of photosynthesis in higher plants by heavy metals has been reported (John *et al.*, 2008). These results are in harmony with those reported by Saleh and Al-Garni, (2006) ; Ahmad *et al.,* (2006). Treated plants with proline concentrations exhibited significant increase in total soluble carbohydrate compared with control treatment .

Conclusion

From the above mentioned results, it could be concluded that, Cd stress had inhibitory effects on the growth and some physiological and antioxidants parameters of *Vicia faba* .Plant treated with different concentration of proline could be contribute to a reduction of the injurious effect of heavy metal stress on growth and some related physiological and antioxidant responses of broad bean (*Vicia faba*) plants.

The results of this experiment have clearly shown proline that could be used effectively to protect *Vicia faba* seedlings from the destructive effects of Cd stress. The effectiveness of proline in inducing Cd stress tolerance depends up on the concentration of proline applied.

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