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# NITROGEN METABOLISM AND ACTIVITY OF ANTIOXIDATIVE ENZYMES IN CHICKPEA PLANTS GROWN IN CADMIUM AMENDED SOILS

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**Abstract**

The present experiment was conducted to elucidate the physio-morphological and biochemical responses of chickpea plants exposed to 0, 25, 50 or 100 mg cadmium (Cd) per kg of soil. Cd was given in the form of $CdCl_2$. It was observed that all the growth parameters (length, fresh and dry mass), number of nodules, their fresh and dry mass were decreased with the increasing concentration of Cd in soil at both the sampling stage i.e. 60 and 90 days after sowing (DAS). The value of leghemoglobin and carbohydrate contents of nodules was also decreased in a concentration dependent manner at 60 and 90 DAS. However, the nitrogen content of the leaf of the plants fed with lowest concentration of cadmium (25 mg/kg of soil) showed a value which is comparable to control. As the level of cadmium increased in the soil, a concomitant reduction in the photosynthetic attributes as well as of leaf nitrogen and root nitrate content was noted. A significant reduction in the activities of nitrate reductase and carbonic anhydrase was also noted and the reduction was more significant in 100 mg Cd fed plants. The enzyme activities in 100 mg Cd fed plants decreased significantly by 37.9%, 38.0% (glutamine synthetase), 28.0%, 29.0% (glutamate synthase) and 46.0%, 44.0% (glutamate dehydrogenase) at two sampling stage (60 and 90 DAS), respectively as compared to control. However, unlike other parameters, the endogenous proline level and the activities of catalase, peroxidase and superoxide dismutase showed an increase with the increasing level of cadmium.

## Introduction

The impact and long-term ecological ramifications of heavy metal pollution on the biosphere have resulted in an increased interest in evaluating the interaction between heavy metals and flora. Of the major heavy metals, cadmium (Cd) is one of the major industrial pollutants (Das *et al.,* 1997). The level of the pollution by cadmium regarded as non-polluted (0-1 mg $kg^{-1}$ of soil), slightly contaminated (1-3 mg $kg^{-1}$ of soil) and highly contaminated (3-10 mg $kg^{-1}$ of soil) (Rodriguez-Flores & Rodriguez-Castellon, 1982). The soil samples collected from the adjoining areas of Aligarh reflected heavy Cd contamination reaching up to 21 mg $kg^{-1}$ of soil (Sharma & Prasad, 2010) which might be due the reason that most of the crops in the region of study fall under the area irrigated with waste water, prone to high Cd accumulation and the excessive use of phosphatic fertilizers (Lu *et al.,* 1992) which may synergize the soil Cd level up to 30 mg $kg^{-1}$ of soil as reported by National Environmental Engineering Research Institute, Roorkee, India (1999-2002). The same study also revealed that the use of municipal solid waste elevates the soil Cd concentration up to 100 mg $kg^{-1}$ of soil (most toxic for plants). Thus a range of high Cd concentration (25 mg per kg soil) to most toxic concentration (100 mg per kg soil) encountered in edaphic conditions has been selected for the study was based on solubility (Yang *et al.,* 2004). It is reported that the function of the chloroplast is altered through the accumulation of cadmium which is due to the inhibition of chlorophyll biosynthesis and fixation of carbon dioxide (Siedlecka *et al.,* 1997). Various physiological processes may be altered, including growth retardation (Bavi *et al.,* 2011; Raziuddin *et al.,* 2011) and plant-water relations (Barcelo & Poschenrieder, 1990; Das *et al.,* 1997). Cd-induced generation of superoxide ($O_2^-$) anions, hydroxyl ($OH^-$) radicals and hydrogen peroxide causes considerable membrane damage. It alters the enzyme activity (Hasan *et al.,* 2009). The aim of this study was to elucidate the degree of damage caused by varying level of Cd and also to investigate the biochemical detoxification strategies that chickpea plant adopts against stress induced by the presence of Cd in soil.

## Material and methods

The seeds of *Cicer arietinum* L. were procured from New Delhi, India. At the start of the experiment, four sets of pots were supplemented with different doses (0, 25, 50 or 100 mg $kg^{-1}$ of soil) of Cd in the form of cadmium chloride. The seeds were sterilized with mercuric chloride (0.01%) solution and inoculated with *Rhizobium ciceri* were sown in these pots. These pots contain farmyard manure and sandy loam soils in the ratio of 1:6. These filled pots were placed in the net house in way to maintain a simple randomized block design. The plants were collected at 60 and 90 days after sowing (DAS) for the study of different parameters.

**Plant growth analysis:** The length of root and shoot of uprooted plants was measured. The fresh mass of plants and nodules were noted and they were dried in an oven (80°C for 72 h) to assess their dry mass. The nodules number per plant was also noted.

**Chemical analysis:** The content of leghemoglobin, carbohydrate, nitrogen and nitrate were estimated as described by Sadasivam & Mannickam (1992), Dubois *et al*., (1956), Lindner (1944) and Singh (1988) respectively. The activity of nitrogenase and nitrate reductase was measured as described by Hardy *et al.,* (1968) and Jaworski (1971) respectively while as that of glutamate synthase, glutamate dehydrogenase, and glutamine synthetase was done as stated by Thimmaiah (1999). The leaf carbonic anhydrase activity was analysed as per the

description of Dwivedi & Randhawa (1974). The water use efficiency, photosynthetic rate, stomatal conductance, internal $CO_2$ concentration and transpiration rate were measured by portable photosynthetic system (LI-COR Lincoln, NE, USA) in the intact leaves. The activities of peroxidase and catalase were measured by the method of Chance & Maehly (1956) while as that of superoxide dismutase by Beauchamp & Fridovich (1971). The leaf proline content was estimated with the procedure of Bates *et al.,* (1973). Each observation was replicated three times. The statistical package SPSS software version 10 (SPSS, Chicago, IL, USA) was used to compared the treatments means. Least significant difference (LSD) was obtained at 5% level of probability. Standard error (±) was also calculated.

## Results

**Growth parameters:** All the growth attributes (length, fresh and dry mass) decreased with increasing doses of Cd at 60 and 90 DAS. A significant drop of 52.5%, 50.0% (root length); 39.9%, 39.5% (shoot length); 55.1%, 50.0% (fresh mass) and 56.7% 55.3% (dry mass) was observed at both the sampling stage, as compare to control, when Cd was supplemented at the rate of 100 mg per kg of soil (Fig. 1).

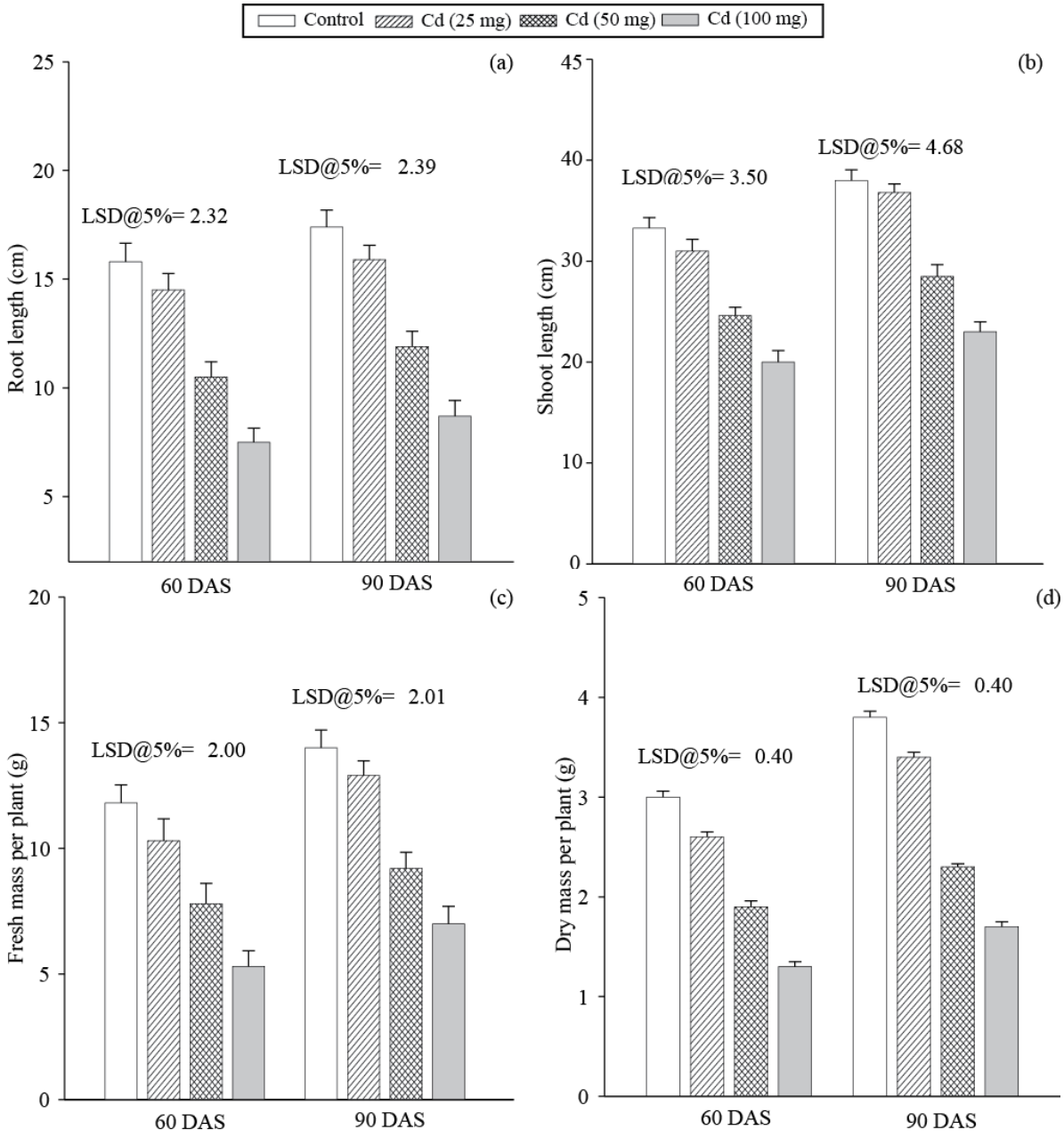


Fig. 1. Effect of Cd on root length (a), shoot length (b), fresh mass (c) and dry mass (d) per plant in *Cicer arietinum* at 60 and 90 days, after sowing (DAS). Data are the mean of three independent replicates. Vertical bars represent standard error (±).

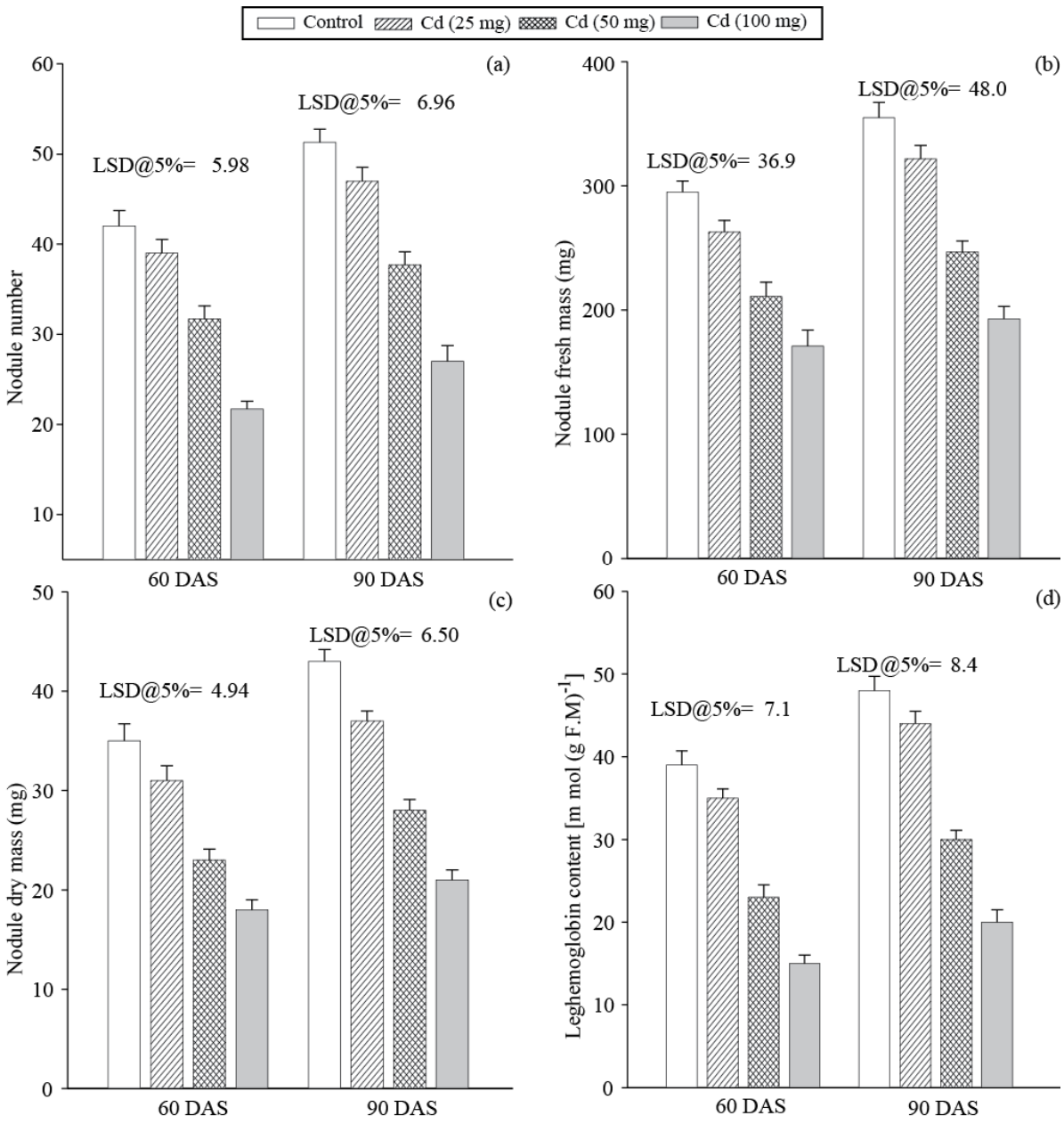


Fig. 2. Effect of Cd on nodule number (a), nodule fresh mass (b), nodule dry mass (c) and leghemoglobin content (d) per plant in *Cicer arietinum* at 60 and 90 days, after sowing (DAS). Data are the mean of three independent replicates. Vertical bars represent standard error (±).

**Nodulation, carbohydrate and leghemoglobin content:** As the age of the plant advance from 60-90 DAS, a substantial increase in the number of nodules, their fresh and dry weight was observed (Fig. 2). However, their values decreased with increasing doses of cadmium. 100 mg Cd per kg of soil proved to be the most toxic and significantly reduced the values of nodule number (48.3%, 47.4%), nodule fresh mass (42.0%, 45.6%) and nodule dry mass (48.6%, 51.2%) at both the sampling stage, in comparisons to control. A similar pattern of response was followed by leghemoglobin (Fig. 2) and carbohydrate content (Table 1) as was observed in nodulation parameters.

**Nitrogenase, Glutamine synthetase, Glutamate synthase and Glutamate dehydrogenase activities in nodules:** Irrespective of the treatments, the activity of nitrogenase in fresh nodules increased as the age progressed from 60 to 90 DAS. However, all the Cd concentrations imparted negative effects on the activity when compared to their respective controls (Table 1). A Cd dose of 50 or 100 mg per kg of soil reduced the nitrogenase activity by 16.3%, 16.4% and 27.9%, 29.3% at both the sampling stage, in comparison to control. The enzymes glutamine synthetase, glutamate synthase and glutamate dehydrogenase in the nodules of the plants supplemented with 25 mg Cd per kg of soil was found to

be statistically at par with that of the control at both the sampling stages. However, the activity of these enzymes showed a significant reduction as the concentration of Cd increased from 50 to 100 mg.

**Leaf nitrogen and root nitrate content:** The values of leaf nitrogen in the plants fed with 25 mg Cd per kg of soil were almost comparable to the control at both the sampling stages. However, the presence of Cd at the rate of 50 and 100 mg per kg of soil significantly decreased these values by 19.2%, 23.6%; 28.7% and 34.5%, at both the sampling stage, in comparison to control (Table 2). Irrespective of the concentration of Cd used, the nitrate content in roots showed an increase as the growth progressed from 60 to 90 DAS.

**Nitrate reductase and carbonic anhydrase activities in leaves:** Cadmium exposure also affected the activities of nitrate reductase and carbonic anhydrase (Tables 2-3). The activity of nitrate reductase and carbonic anhydrase were significantly reduced under the influence of 50 and 100 mg Cd per kg of soil reducing the values by 17.8%, 16.9%; 30.1% and 28.6% at 60 and 90 DAS, for nitrate reductase and by 21.4%, 22.0%; 33.8% and 35.2% for carbonic anhydrase, respectively at both the sampling stage.

**Table 1. Effect of Cd on carbohydrate content (%), nitrogenase [n mol $C_2H_5$ (g nodule FM)$^{-1}$], glutamine synthetase [µ mol γ GH (gFM)$^{-1}h^{-1}$] and glutamate synthase [µ mol NADHox (gFM)$^{-1}h^{-1}$] activities in the nodules of *Cicer arietinum* at 60 and 90 DAS (± = S.E.).**

| Treatments | Carbohydrate content | | Nitrogenase activity | | Glutamine synthetase activity | | Glutamate synthase activity | |
|---|---|---|---|---|---|---|---|---|
| | 60 DAS | 90 DAS | 60 DAS | 90 DAS | 60 DAS | 90 DAS | 60 DAS | 90 DAS |
| Control | 14.90±0.4 | 17.00±0.5 | 380±7.57 | 403±6.24 | 285±5.5 | 322±3.6 | 139±3.8 | 148±3.8 |
| Cd (25 mg per kg soil) | 13.70±0.3 | 15.98±0.8 | 362±5.86 | 383±5.51 | 257±7.0 | 295±5.5 | 133±2.6 | 140±3.8 |
| Cd (50 mg per kg soil) | 11.82±0.3 | 13.80±0.1 | 318±6.81 | 337±7.23 | 223±4.7 | 250±4.3 | 114±2.9 | 121±3.2 |
| Cd (100 mg per kg soil) | 9.40±0.5 | 11.00±0.2 | 274±7.81 | 285±6.93 | 177±4.3 | 200±3.2 | 100±2.3 | 105±2.3 |
| LSD @ 0.05 | 1.5 | 1.3 | 31.00 | 34.27 | 30 | 29 | 9 | 10 |

**Table 2. Effect of Cd on nodule glutamate dehydrogenase activity [µ mol NADHox (gFM)$^{-1}h^{-1}$], root nitrate content (mg g$^{-1}$ DM), leaf nitrogen content (%) and nitrate reductase activity [n mol $NO_2$ h$^{-1}$g$^{-1}$FM] of *Cicer arietinum* at 60 and 90 DAS (± = S.E.)**

| Treatments | Glutamate dehydrogenase activity | | Nitrate content | | Leaf nitrogen content | | Nitrate reductase activity | |
|---|---|---|---|---|---|---|---|---|
| | 60 DAS | 90 DAS | 60 DAS | 90 DAS | 60 DAS | 90 DAS | 60 DAS | 90 DAS |
| Control | 31.0±0.6 | 35.0±0.8 | 150±4.3 | 165±6.1 | 3.13±0.04 | 4.20±0.08 | 292±6.2 | 385±7.0 |
| Cd (25 mg per kg soil) | 29.1±0.5 | 33.8±0.4 | 141±3.0 | 153±5.3 | 2.94±0.04 | 3.99±0.06 | 274±6.4 | 365±5.7 |
| Cd (50 mg per kg soil) | 22.3±0.3 | 25.0±0.3 | 114±2.6 | 130±4.2 | 2.53±0.06 | 3.21±0.04 | 240±5.8 | 320±6.6 |
| Cd (100 mg per kg soil) | 16.7±0.3 | 19.6±0.4 | 100±2.6 | 110±4.2 | 2.20±0.10 | 2.75±0.08 | 204±6.1 | 275±6.4 |
| LSD @ 0.05 | 3.5 | 2.5 | 14.4 | 16 | 0.20 | 0.32 | 24.8 | 30 |

**Table 3. Effect of Cd on carbonic anhydrase activity [mol ($CO_2$) kg$^{-1}$ leaf (F.M)], stomatal conductance (mol m$^{-2}$ sec$^{-1}$), internal carbon dioxide concentration (ppm) and water use efficiency in *Cicer arietinum* at 60 and 90 DAS (± = S.E.).**

| Treatments | Carbonic anhydrase activity | | Stomatal conductance | | Internal carbon dioxide concentration | | Water use efficiency | |
|---|---|---|---|---|---|---|---|---|
| | 60 DAS | 90 DAS | 60 DAS | 90 DAS | 60 DAS | 90 DAS | 60 DAS | 90 DAS |
| Control | 2.99±0.09 | 3.24±0.03 | 0.311±0.002 | 0.345±0.003 | 290±2.6 | 316±2.6 | 0.38±0.006 | 0.42±0.01 |
| Cd (25 mg per kg soil) | 2.71±0.04 | 2.94±0.05 | 0.295±0.002 | 0.291±0.003 | 273±2.6 | 295±2.3 | 0.32±0.02 | 0.37±0.01 |
| Cd (50 mg per kg soil) | 2.35±0.07 | 2.53±0.07 | 0.227±0.001 | 0.239±0.003 | 240±1.7 | 266±3.4 | 0.20±0.10 | 0.28±0.06 |
| Cd (100 mg per kg soil) | 1.98±0.04 | 2.10±0.06 | 0.200±0.002 | 0.215±0.002 | 205±2.3 | 220±3.2 | 0.13±0.01 | 0.19±0.01 |
| LSD @ 0.05 | 0.29 | 0.32 | 0.02 | 0.02 | 24 | 26 | 0.06 | 0.06 |

**Photosynthetic parameters:** All the photosynthetic parameters such as stomatal conductance, internal carbon dioxide concentration, water use efficiency, transpiration rate and photosynthetic rate decreased substantially with the increasing concentration of cadmium in the soil. The value of these parameters decreased significantly as the concentration of cadmium was increased in soil where maximum significant reduction of 35.7%, 37.7% (stomatal conductance); 29.3%, 30.4% (internal carbon dioxide concentration); 65.8%, 54.8% (water use efficiency); 55.9%, 52.3% (transpiration rate) and 41.8%, 45.0% (photosynthetic rate) at both the stage of sampling, was observed in the plants exposed to 100mg Cd per kg of soil.

**Antioxidative enzyme activities and proline content:** The activities of antioxidative enzymes as well as that of proline increased with increasing concentration of Cd and with the age of plant (Tables 4-5). Exposure to 50mg Cd per kg of soil significantly increased the values by 15.1%, 17.8% (Catalase); 26.9%, 40.5% (Peroxidase); and 18.5%, 23.7% (Super oxide dismutase) at both the stage. Activities of catalase, peroxidase and superoxide dismutase were further elevated by 100 mg Cd per kg of soil. Whereas, the increase was not significant when Cd was supplemented at the rate of 25mg per kg of soil (Tables 4-5).

**Table 4. Effect of Cd on transpiration (m mol $m^{-2}$ $sec^{-1}$), photosynthetic rate (m mol $CO_2$ $m^{-2}$ $sec^{-1}$), catalase activity [μ mol $H_2O_2$ decomposed (g FM)$^{-1}$] and peroxidase activity [units (g$^{-1}$FM)] activity in *Cicer arietinum* at 60 and 90 DAS (± = S.E.).**

| Treatments | Transpiration | | Photosynthetic rate | | Catalase activity | | Peroxidase activity | |
|---|---|---|---|---|---|---|---|---|
| | 60 DAS | 90 DAS | 60 DAS | 90 DAS | 60 DAS | 90 DAS | 60 DAS | 90 DAS |
| Control | 1.68±0.01 | 1.91±0.01 | 6.50±0.1 | 7.40±0.02 | 403±4.7 | 438±4.7 | 14.5±1.12 | 16.3±0.15 |
| Cd (25 mg per kg soil) | 1.50±0.01 | 1.67±0.01 | 5.85±0.02 | 6.55±0.03 | 419±4.9 | 457±5.5 | 15.3±0.17 | 18.0±0.20 |
| Cd (50 mg per kg soil) | 1.01±0.01 | 1.22±0.01 | 4.52±0.04 | 5.10±0.03 | 464±6.6 | 516±5.8 | 18.4±0.15 | 22.9±0.30 |
| Cd (100 mg per kg soil) | 0.74±0.07 | 0.91±0.01 | 3.78±0.02 | 4.07±0.03 | 502±5.7 | 567±4.1 | 20.3±0.12 | 25.8±0.20 |
| LSD @ 0.05 | 0.20 | 0.26 | 0.70 | 0.92 | 27.93 | 28.98 | 1.3 | 2.1 |

**Table 5. Effect of Cd on superoxide dismutase [units (g$^{-1}$FM)] activity and proline content [mg (g FM)$^{-1}$] İn *Cicer arietinum* at 60 and 90 DAS (± = S.E.).**

| Treatments | Superoxide dismutase | | Proline content | |
|---|---|---|---|---|
| | 60 DAS | 90 DAS | 60 DAS | 90 DAS |
| Control | 135±2.1 | 156±2.1 | 9.52±0.14 | 11.00±0.13 |
| Cd (25 mg per kg soil) | 142±2.1 | 169±1.7 | 10.50±0.10 | 13.15±0.13 |
| Cd (50 mg per kg soil) | 160±1.7 | 193±2.1 | 11.80±0.15 | 14.85±0.17 |
| Cd (100 mg per kg soil) | 178±1.7 | 219±2.1 | 13.20±0.15 | 16.71±0.14 |
| LSD @ 0.05 | 12.74 | 18.98 | 1.00 | 0.9 |

## Discussion

A large number of metabolites mainly proline was accumulated in the plants, when they grow under various abiotic stress (Ashraf & Foolad, 2007). Such an accumulation of proline in plants was also observed in this study (Table 5) when exposed to Cd stress in a concentration dependent manner. A plausible reason might be that proline acts as an excellent osmolyte and is known for stabilizing sub-cellular structures such as proteins and cell membranes, scavenging free radicals and buffering redox potential under abiotic stress (Ashraf & Foolad, 2007). Cadmium causes a number of effects on the plant which may be direct or indirect on growth and metabolism (Hasan *et al.,* 2009; Ahmad *et al.,* 2012) by forming different complexes with oxygen, nitrogen and sulphur ligands (Van Assche & Clijsters, 1990).

In this study, exposure of plants to increasing concentration of Cd resulted in reduction of growth (Fig. 1). The probable reason for this decrease might be that Cd becomes associated with the cell wall and middle lamellae, which increases the cross linking of pectins and results in the inhibition of cell growth (Poschenrieder *et al.,* 1989). Cd brings about the closure of stomata by decreasing the partial pressure of carbon dioxide in the stroma (Barcelo & Poschenreider, 1990) which becomes a direct cause of reducing the stomatal conductance, internal carbon dioxide concentration, water use efficiency and transpiration rate (Tables 3-4). Further, it is added that the activity of chlorophyllase is increased by the application of cadmium which leads to the degradation of chlorophyll (Reddy & Vora, 1986) and also decrease the formation of δ-amino-levulinic acid (Gadallah, 1995) leading to reduced pigment concentration (Larsen *et al*., 1998) which ultimately reduced the photosynthesis (Table 4).The reduced photosynthesis in Cd treated plants is also be due to the reduced activity of carbonic anhydrase (Table 3).

Carbonic anhydrase facilitates the diffusion of carbon dioxide across the chloroplast membrane by catalyzing the hydration of dissolved carbon dioxide as it enters the most alkaline environment of stroma (Majeau & Coleman, 1994) where carbonic anhydrase catalyzes the reversible hydration of carbon dioxide and maintains a constant supply of RuBPCO. The reported decrease in the carbonic anhydrase activity by Cd is in agreement with others (Hasan *et al.,* 2009). The activity of this enzyme is mainly determined by various factors such as zinc availability, level of carbon dioxide and density of photon flux (Tiwari *et al*., 2005). The partial pressure of carbon dioxide in the stroma is decreased by cadmium through inducing the closure of stomata (Barcelo & Poschenreider, 1990) which may result in the loss of carbonic anhydrase activity. The decreased net photosynthetic rate coupled with reduced carbonic anhydrase activity resulted in the production and accumulation of photosynthates thereby decreasing the carbohydrate content in nodules (Table 1)

The activity of nitrate reductase decreased in the plants of the leaves which are exposed to foliar applied cadmium (Table 2). It may be due to an inhibition in the protein (Hopkins, 1995). Obata *et al*., (1996) also emphasized the negative impact of the metal on the activity of plasma membrane bound ATP proton pump which may influence the fluidity of membranes (Meharg, 1993) leads to restrict the uptake of nitrate, an inducer of nitrate reductase (Campbell, 1999) thereby decreasing the nitrate content in roots (Table 2) and consequently the activity of nitrate reductase (Table 2).

In the natural condition when the plants are exposed to any stress reactive oxygen species are generated in a huge quantity (Schutzendubell & Polle, 2002) which may oxidise lipids, proteins and nucleic acids, leading to the abnormalities in the cell (Sania di toppi *et al*., 1998). The plants have capability to counter such stress by antioxidants such as proline (Table 5) and enzymes (catalase, peroxidase and superoxide dismutase) (Tables 4-5) that give more power of resistance to neutralize the toxic effects of the stress generated through reactive oxygen species (Schutzendubel & Polle, 2002) such as superoxide radical, hydroxyl ions and hydrogen peroxide, therefore, may be regarded as the defense strategy adopted by the plants to counter stress.

The sequence that leads to the establishment of nitrogen fixing mature nodules begins with the infection thread formed by the joint action of bacteria and host and nitrogen fixing potential of each nodule is determined by three main factors (Marschner, 2003); (a) photosynthate availability (b) low oxygen supply to the bacteroid, which at excessive level inhibits nitrogenase (c) export of fixed nitrogen in the form of ammonia. Nitrogen fixed in the form of ammonia diffuses across the peribacteroid membrane to the host cytosol by simple diffusion (Udvardi & Day, 1990). Here two enzymatic systems, (a) glutamate dehydrogenase and (b) glutamine synthetase, glutamate synthaseare operative to further metabolize it. Glutamate dehydrogenase causes direct reductive amination of α-ketoglutarate, giving glutamate, whereas, glutamine synthetase catalyses the addition of ammonia to glutamate forming corresponding amide, glutamine. This glutamine is converted back to glutamate by transfer of amide group to a molecule of α-ketoglutarate (Hopkins, 1995). Cadmium is known to decrease nodulation (Rana & Ahmad, 2002) by affecting the establishment of symbiosis between host and *Rhizobium*, thereby decreasing nodulation expressed in terms of decreased number, fresh and dry mass of nodules (Fig. 2) and ultimately reducing the activity of enzyme nitrogenase (Table 1) and consequently total nitrogen content (Table 2). Cd stress is also known to induce nodule senescence (Balestrasse *et al.,* 2004) thereby decreasing the nodulation (Fig. 2) and leghemoglobin content (Fig. 1) which protects the oxygen labile enzyme nitrogenase, thereby decreasing its activity as well. The leghemoglobin breakdown might be a result of increased generation of reactive oxygen species which is a characteristic feature of Cd toxicity (Balestrasse *et al.,* 2004). The increasing concentration of Cd in soil inhibits the activity of the enzymes of nitrogen assimilation glutamine synthetase, glutamate synthase and glutamate dehydrogenase (Tables 1-2) either by interfering the transcription and/or translation (Prasad, 1995) or through the increased generation of reactive oxygen species (Hasan *et al.,* 2009).

## Conclusion

In the present investigation, out of three different concentrations of Cd (25, 50 or 100 mg per kg) applied through soil, 100 mg proved to be most toxic. The decrease in morphological, photosynthetic parameters and activity of enzymes (nitrate reductase, carbonic anhydrase, nitrogenase, glutamine synthetase, glutamate synthase and glutamate dehydrogenase) is depend on the concentration of the cadmium in the soil. The activity of antioxidant enzymes (catalase, peroxidase and superoxide dismutase) and the proline content showed an increase with the increasing concentration of Cd in soil. This increase in defense to counter stress is attributed to enhanced activity of enzymes (catalase, peroxidase and superoxide dismutase) adopted by plants.

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