Cadmium is a highly toxic, metallic soil contaminant, having no metabolic use, which adversely affects the plant growth, especially at early stages and has resulted in the loss of crop productivity. Cadmium has an increasing international concern because of its toxicity and is generally considered to be much higher than those of other heavy metals. The present study was carried out in order to evaluate the effects of Cadmium stress on biochemical parameters such as chlorophyll content, protein content and peroxidase enzyme activity for 7 days old seedlings of different varieties (Virat, SML-668, A1 Gold and K-851) of mung bean (Vigna radiata). Cadmium caused significant (p<0.05) reduction in chlorophyll and protein content. Peroxidase activity was increased significantly (p<0.05) with the increasing Cadmium concentration. Reduction of chlorophyll content is due to the poor growth of seedlings under stress condition. Decreased protein content was due to an enhanced rate of protein degradation, while increased peroxidase activity was indicative of Cadmium induced oxidative stress in V. radiata.

Keywords : Vigna radiata, chlorophyll content, protein content, peroxidase activity, oxidative stress.

Introduction

Among the heavy metals, Cadmium (Cd) has an increasing international concern due to its toxicity and is generally considered to be much higher than those of other heavy metals and it is readily taken up by plants (Sarkar, 2002). Higher concentration of cadmium in soils represents a potential threat to human health. It is incorporated into the food chain mainly by plant uptake (Alvarez, 2008). Cadmium causes oxidative stress in plants and has a high level of toxicity in plants, animals and human. A high cadmium level causes some biochemical changes in the plants such as production of reactive oxygen species (ROS) i.e. superoxide, hydrogen peroxide and hydroxyl radicals that lead to oxidative stress (Cho and Park, 2000). These ROS cause oxidative damage in plants by lipid peroxidation, membrane damage and consequently in plant senescence (Zhang et al., 2003) and also leads to protein degradation, breaking of DNA and cell death. Mostafa and Sarani (2011) showed that the activity of antioxidant enzymes are elevated with increased Cadmium concentration.

V. radiata (mung) is the most important legume of the family Fabaceae. It is one of the most widely used pulse crop in India. It has great value as food, and is a cheap source of protein for direct human consumption (Mubarak, 2005). The aim of the present study was to investigate the impact of Cadmium stress on chlorophyll content, protein content and peroxidase activity in 4 different varieties (Virat, SML-668, A1 Gold and K-851) of V. radiata seedlings.
Materials and methods

The experiments were carried out in the laboratory of Department of Biochemistry at Govt. Holkar Science College, Indore from December 2011 to May 2012. Four different varieties of mung bean seeds (*Vigna radiata*) viz., Virat, SML-668, A1 Gold and K- 851 were chosen for the experimental work. The seeds were purchased from Sachchidanand Krashi Seva Kendra, Nandlalpura, Indore and Manish Traders, seed depot, Tejaji Nagar, Indore. Seeds of *V. radiata* were germinated for 7 days. During the experiments, all varieties were given the same treatment. CdCl$_2$ with concentrations 50, 100, 150, 200 and 250 µM were used for the treatment.

Seeds surface sterilization and treatment process

Prior to germination, seeds were surface sterilized with 0.1% mercuric chloride (HgCl$_2$) solution for 5 minutes (Vijayaragavan *et al.*, 2011) to avoid fungal contamination. Seeds were then washed thoroughly for 4 - 5 times with sterilized distilled water (Yun Shao *et al.*, 2011). After sterilization, the uniform and healthy seeds were selected for germination and were placed in 10 cm diameter Petri dishes lined with Whatman No. 1 filter paper (Yaqvob *et al.*, 2011). Seeds were cultured in each Petri dish with sterilized distilled water for 24 hours at room temperature (25±3°C) in dark. The criterion used for seed germination was taken as emergence of 1 mm radicle at the time of observation (Sharma *et al.*, 2011).

After 24 hours of germination, fifteen germinated seeds with desired criteria were evenly transferred to each Petri dish (diameter 10 cm) lined with Whatman No. 1 filter paper. Seeds were arranged in such a way neither touched each other nor touched the side of the dish. The Petri dishes were treated with an equal volume of six different concentrations of CdCl$_2$ solutions (0, 50, 100, 150, 200 and 250 µM). At the start of experiment, 3 ml of respective treatment was added in order to moisten the filter paper in each Petri dish and at every alternate day 2 ml of respective treatment was added. Control sets were run with seeds kept in distilled water. Each treatment including the control was replicated three times for reliability. All the Petri dishes were kept at room temperature (25±3°C) in dark.

Estimation of chlorophyll content

Fresh leaf samples were extracted with 80% acetone. For spectrophotometric determination of chlorophyll a, chlorophyll b and total chlorophyll contents, the absorbance of the extracts were measured at 645 and 663 nm, respectively following the method by Sadasivam and Manickam (1992).

Estimation of Protein content

Method used for Protein estimation was Biuret method (Jayaraman, 1981). It is based on the fact that the -CO-NH- groups (peptide bonds) of protein form a purple complex with copper ions in an alkaline solution. The intensity of the purple complex is measured at 520 nm colorimetrically.

Estimation of Peroxidase activity

The enzyme activity was assayed using o-dianisidine as hydrogen donor and H$_2$O$_2$ as electron acceptor. The rate of formation of yellow orange colored dianisidine dehydrogenation product is a measure of peroxidase activity and can be assayed spectrophotometrically at 430nm in terms of units /min /g according to Summer and Gjessing *et al.* (1943).

Statistical analysis

Chlorophyll content, protein content and peroxidase activity of *V. radiata* under different concentrations of Cadmium Chloride were expressed as mean ± standard deviation (SD). ANOVA was used to compare parameters of control vs treated seedlings. P values less than 0.05 was considered to be significant.
Results and discussion

Clorophyll content

Chlorophyll a, Chlorophyll b and total Chlorophyll content of the leaves of seedlings were decreased at all levels of CdCl₂ treatment in all studied varieties (Virat, SML-668, A1 Gold and K-851) of Vigna radiata. However, this decrease was found to be insignificant with 50 µM conc. and was observed to be (p<0.06), (p<0.10) and (p<0.09) for Chlorophyll a, Chlorophyll b and total Chlorophyll content respectively as compared to untreated seedlings. The seedlings treated with 100 µM conc. of CdCl₂ shows a significant decrease (p<0.02) in both Chlorophyll b and total Chlorophyll content, while Chlorophyll a was found to be very significantly (p<0.004) decreased in all varieties. Highly significant (p<0.01) decrease was observed above 150 µM concentration in all the studied parameters in all varieties. The maximum reduction was observed in Virat variety of V. radiata at 250 µM concentration of CdCl₂ i.e. (71.65%), (85.60%) and (81.25%) in Chlorophyll a, Chlorophyll b and total Chlorophyll respectively (Table - 1), although these parameters were least affected in SML-668 variety at the same concentration (Table - 2). Decreased chlorophyll content associated with Cadmium heavy metal stress may be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis. Various abiotic stresses decrease the chlorophyll content in plants (Ahmad et al., 2007). The reduction in Chlorophyll a, chlorophyll b and total chlorophyll content due to Cadmium stress has also been reported by John et al. (2008) in Lemna polyrrhiza L. Photosynthetic pigments such as chlorophyll - a, chlorophyll-b, and total chlorophyll content of cowpea leaves were decreased with increase in Cadmium level. The loss in chlorophyll content can consequently lead to disruption of photosynthetic machinery (Vijayaragavan et al., 2011).

Protein content

The protein content in 50 µM treated seedlings of Virat, SML-668, A1 Gold and K-851 was insignificantly

Table - 1. Table showing values for different parameters in untreated vs treated 7 days old seedlings in virat variety of V. radiate

<table>
<thead>
<tr>
<th>CdCl₂ concentration (µM)</th>
<th>Chlorophyll - a content (mg/gm)</th>
<th>Chlorophyll - b content (mg/gm)</th>
<th>Total Chlorophyll content (mg/gm)</th>
<th>Protein content (gm)</th>
<th>Peroxidase activity (units/min/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.27 ± 0.02 (12.26%)</td>
<td>1.32 ± 0.02 (24.24%)</td>
<td>1.28 ± 0.01 (17.97%)</td>
<td>0.30 ± 0.02</td>
<td>0.62 ± 0.01 (6.06%)</td>
</tr>
<tr>
<td>50</td>
<td>1.00 ± 0.04 ns (32.28%)</td>
<td>1.00 ± 0.03 ns (34.9%)</td>
<td>1.05 ± 0.02 ns (33.59%)</td>
<td>0.24 ± 0.03 ns (20%)</td>
<td>0.66 ± 0.02 ns (16.22%)</td>
</tr>
<tr>
<td>100</td>
<td>0.86 ± 0.02 * (32.28%)</td>
<td>0.87 ± 0.02 * (34.9%)</td>
<td>0.85 ± 0.01 * (33.59%)</td>
<td>0.18 ± 0.02 * (40%)</td>
<td>0.74 ± 0.01 * (16.22%)</td>
</tr>
<tr>
<td>150</td>
<td>0.75 ± 0.03 ** (40.94%)</td>
<td>0.64 ± 0.02 ** (51.52%)</td>
<td>0.64 ± 0.01 ** (50%)</td>
<td>0.14 ± 0.03 ** (53.35%)</td>
<td>0.79 ± 0.01 ** (21.52%)</td>
</tr>
<tr>
<td>200</td>
<td>0.46 ± 0.04 ** (63.78%)</td>
<td>0.43 ± 0.02 ** (67.42%)</td>
<td>0.43 ± 0.01 ** (66.41%)</td>
<td>0.13 ± 0.02 ** (56.67%)</td>
<td>0.92 ± 0.02 ** (32.61%)</td>
</tr>
<tr>
<td>250</td>
<td>0.36 ± 0.02 ** (71.65%)</td>
<td>0.19 ± 0.02 ** (85.60%)</td>
<td>0.24 ± 0.01 ** (81.25%)</td>
<td>0.10 ± 0.02 ** (66.67%)</td>
<td>1.07 ± 0.03 ** (42.06%)</td>
</tr>
</tbody>
</table>

ns = not significant, * and ** = significant at p<0.05 and p<0.01 respectively. Values in brackets showed percent reduction/increase as compared to untreated seedlings.
(p<0.16) decreased as compared to untreated seedlings, while those treated with 250 µM showed a significant (p<0.0002) decrease as compared to untreated seedlings and the maximum reduction (66.67%) was observed in Virat and SML-668 variety (Table -1 and 2). These results supported the study of Verma et al. (2012) who showed that soluble protein content was decreased in seedlings with increasing concentration of Cadmium Chloride over the control seedlings. Results of John et al. (2008) showed that Cadmium treatment (20 mg/l) was resulted in reduction of soluble protein in L. polyrrhiza. Mohan and Hosetti et al., (1997) found more

### Table - 2. Table showing values for different parameters in untreated vs treated 7 days old seedlings in SML - 668 variety of V. radiata

<table>
<thead>
<tr>
<th>CdCl₂ conc. (µM)</th>
<th>Chlorophyll-a content (mg/gm)</th>
<th>Chlorophyll-b content (mg/gm)</th>
<th>Total Chlorophyll content (mg/gm)</th>
<th>Protein content (gm)</th>
<th>Peroxidase activity (units/min/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.38 ± 0.01</td>
<td>2.02 ± 0.04</td>
<td>1.89 ± 0.01</td>
<td>0.33 ± 0.02</td>
<td>0.74 ± 0.02</td>
</tr>
<tr>
<td>50</td>
<td>1.26 ± 0.02*</td>
<td>1.76 ± 0.02*</td>
<td>1.66 ± 0.02*</td>
<td>0.31 ± 0.03</td>
<td>0.82 ± 0.02*</td>
</tr>
<tr>
<td>100</td>
<td>1.14 ± 0.02*</td>
<td>1.55 ± 0.01*</td>
<td>1.46 ± 0.03*</td>
<td>0.23 ± 0.02</td>
<td>0.95 ± 0.02*</td>
</tr>
<tr>
<td>150</td>
<td>0.95 ± 0.02*</td>
<td>1.34 ± 0.02*</td>
<td>1.25 ± 0.03*</td>
<td>0.21 ± 0.01</td>
<td>1.05 ± 0.03*</td>
</tr>
<tr>
<td>200</td>
<td>0.78 ± 0.01</td>
<td>1.13 ± 0.02</td>
<td>1.02 ± 0.02</td>
<td>0.16 ± 0.01</td>
<td>1.13 ± 0.02*</td>
</tr>
<tr>
<td>250</td>
<td>0.69 ± 0.02</td>
<td>0.87 ± 0.01</td>
<td>0.84 ± 0.01</td>
<td>0.11 ± 0.02</td>
<td>1.29 ± 0.01*</td>
</tr>
</tbody>
</table>

ns = not significant, * and ** = significant at p<0.05 and p<0.01 respectively. Values in brackets showed percent reduction/increase as compared to untreated seedlings.

### Table – 3. Table showing values for different parameters in untreated vs treated 7 days old seedlings in A-1 Gold variety of V. radiata

<table>
<thead>
<tr>
<th>CdCl₂ conc. (µM)</th>
<th>Chlorophyll-a content (mg/gm)</th>
<th>Chlorophyll-b content (mg/gm)</th>
<th>Total Chlorophyll content (mg/gm)</th>
<th>Protein content (gm)</th>
<th>Peroxidase activity (units/min/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.34 ± 0.01</td>
<td>1.57 ± 0.02</td>
<td>1.50 ± 0.01</td>
<td>0.41 ± 0.02</td>
<td>0.33 ± 0.01</td>
</tr>
<tr>
<td>50</td>
<td>1.13 ± 0.02*</td>
<td>1.31 ± 0.02*</td>
<td>1.26 ± 0.02*</td>
<td>0.36 ± 0.02</td>
<td>0.60 ± 0.02*</td>
</tr>
<tr>
<td>100</td>
<td>0.98 ± 0.02*</td>
<td>1.08 ± 0.04*</td>
<td>1.05 ± 0.02*</td>
<td>0.34 ± 0.02</td>
<td>0.73 ± 0.02*</td>
</tr>
<tr>
<td>150</td>
<td>0.83 ± 0.02*</td>
<td>0.86 ± 0.02*</td>
<td>0.85 ± 0.03*</td>
<td>0.30 ± 0.01</td>
<td>0.81 ± 0.02*</td>
</tr>
<tr>
<td>200</td>
<td>0.69 ± 0.03*</td>
<td>0.63 ± 0.02*</td>
<td>0.64 ± 0.03*</td>
<td>0.24 ± 0.01</td>
<td>0.87 ± 0.02*</td>
</tr>
<tr>
<td>250</td>
<td>0.59 ± 0.03*</td>
<td>0.45 ± 0.01</td>
<td>0.44 ± 0.02*</td>
<td>0.19 ± 0.01</td>
<td>1.01 ± 0.03*</td>
</tr>
</tbody>
</table>

ns = not significant, * and ** = significant at p<0.05 and p<0.01 respectively. Values in brackets showed percent reduction/increase as compared to untreated seedlings.
pronounced decrease in the protein content with Cadmium as compared to lead treatment in L. minor. The decrease in protein content in L. polyrrhiza was caused by enhanced protein degradation process as a result of increased protease activity (Palma et al., 2002) that was found to increase under stress conditions.

**Peroxidase activity**

The peroxidase activity was enhanced in the seedlings subjected to Cadmium stress. This increase was found to be insignificant (p<0.05) at 50 µM conc. of CdCl₂, significant (p<0.04) at 100 µM conc. and highly significant (p<0.01) above 150 µM of CdCl₂ as compared to untreated seedlings The untreated seedlings of A1 Gold variety showed a minimum peroxidase activity as compared to other varieties. Maximum increase (67.33%) was observed in A1 Gold variety at 250 µM (Table - 3). The higher level of peroxidase activity was indicative of the increased antioxidative activity under Cadmium stress. These results supported the conclusions of Mostafa and Semin (2011) who reported an increase in activity of antioxidant enzymes such as ascorbate peroxidase, guaiacol peroxidase and catalase in Mustard (Sinapis arvensis L.) plants with increased Cadmium concentration.

**Conclusion**

It can be concluded that cadmium induced oxidative stress and Vigna radiate seedlings were able to enhance the activity of antioxidant enzyme. Among all the varities the Virat variety was found to be most sensitive and easily susceptible for oxidative stress.

**Acknowledgment**

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**References**


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