Effect of Cadmium exposures on growth and biochemical parameters of *Vigna radiata* seedlings

Mamta Hirve, Angoorbala Bafna
1- Department of Biochemistry, Govt. Holkar Science College, Indore, (M.P), INDIA
mamtahirve@gmail.com
doi: 10.6088/ijes.2013040300009

ABSTRACT

Cadmium is one of the toxic heavy metals present in soil as a result of use of insecticides, fungicides and commercial fertilizers and also present in sludge which is used for irrigation. An attempt has been made to assess the response of germinating seedling of Mung bean (*Vigna radiata*) under influence of Cadmium Chloride (CdCl₂) with special reference to growth, morphology and biochemical aspects. In the present study the effect of different concentrations of Cadmium Chloride on the growth parameters such as, root and shoot length, fresh and dry weight and biochemical constituents such as protein and carbohydrate content of 7 days old seedlings were estimated and compared with the parameters of untreated seedlings. The study results revealed that Cadmium adversely and significantly (p<0.05) influenced the growth parameters as well as biochemical constituents of seedling on increasing the concentration of Cadmium.

Keywords: Cadmium Chloride, growth parameters, *Vigna radiata*, protein content, carbohydrate content.

1. Introduction

Heavy metals pollution is widespread, due to rapid industrialization and current agricultural practices. These pollutants persist in the environment for a longer period of time, as they are not easily degraded by soil microorganisms and therefore, can easily be absorbed by plants (Gallego JLR et al, 2002). Heavy metals that have been absorbed by the plants results in growth inhibition, increasing senescence which leads to decreasing crop yield. Moreover, they can be accumulated in the crops affecting human health upon consumption (Zhang Q et al, 2007).

Among heavy metals Cadmium is one of the toxic elements that have no function in living organisms. It has long biological persistence causes leaf rolls chlorosis, growth inhibition, water imbalance, phosphorus and nitrogen deficiency, reduced manganese transport and reduction of root and stem growth (Mishra S et al, 2006). It can be found in soils because it is present in insecticides, fungicides, sludge and commercial fertilizers (Ravichandran S et al, 2011). Cadmium treatment with 1μM in 24 hours reduced the root-growth up to 30% and this inhibition had positive correlation with the reduction of root cells viability (Siroka B et al, 2004). It is widely recognized that Cadmium taken up by plants is the main source of Cadmium accumulation in food (Lopez-Millan AF et al, 2009). Cadmium can be easily absorbed by plant roots and transported to shoots results in disorders in biochemical and physiological processes, and then affects plant growth and morphology (Sgherri C et al, 2002). It has been suggested that growth inhibition by Cadmium is due to a direct effect of Cadmium on the nucleus or its interaction with hormones in the aerial parts of the plants. It also adversely affects photosynthesis (Laspin NV et al, 2005).
V. radiata (mung) is a plant 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 A.E., 2005). The present study was carried out with the aim to observe impact of different Cadmium levels on growth as well as biochemical parameters of 7 day old seedlings of V. radiata and to provide a theoretical basis for the risk assessment of heavy metal pollution and the maintenance of sustainable agricultural production.

2. Materials and methods

The experimental work of the study was carried out in the laboratory of Department of Biochemistry, Govt. Holkar Science College, Indore, during December 2011 - May 2012. Four different varieties of mung bean seeds (Vigna radiata) viz., Virat, SML-668, A1 Gold and K- 851 were selected 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 have given the same treatment. CdCl$_2$ with concentrations 50, 100, 150, 200 and 250 µM were used for the treatment.

2.1 Seeds surface sterilization and treatment process

Prior to germination, seeds were surface-sterilized with 0.1% mercuric chloride for 5 minutes (Vijayaragavan M et al, 2011) to avoid fungal contamination. Seeds were then washed thoroughly for 4-5 times with sterilized distilled water (Shao Y 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 (Mami Y et al, 2011). Seeds were cultured in each Petri dish with sterilized distilled water for 24 hours at room temperature (25±3ºC) in dark. The criterion used for seed germination was taken as emergence of 1 mm radicle at the time of observation (Sharma A et al, 2011).

After 24 hours of germination, 15 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 that they neither touch each other nor touch 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±3ºC) in dark.

2.2 Measurement of root and shoot length

Shoot length was measured from culms base to the tip of the longest leaf, and root length was measured from the root-shoot junction to the tip of the longest root after 7 days of germination. The root and shoot lengths were measured in centimeter using a meter rule.

2.3 Measurement of fresh and dry weight

The fresh weight was measured with an electrical weighing balance. Thereafter, the Petri-dishes containing the fresh seedlings were placed in a hot air oven at 80°C for 24 hours for determination of dry weight of seedlings. The dry matter of seedlings was measured with electrical balance.
2.4 Estimation of total carbohydrate content

Phenol sulphuric acid method (Sadasivam S., and Manickam A., 1992) was used for estimation of total carbohydrates. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This forms an orange-yellow colored product with phenol which has absorption maximum at 490 nm.

2.5 Estimation of protein content

Protein estimation was done by Biuret method (Jayaraman, J. 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.

2.6 Statistical analysis

All the studied parameters of *V. radiata* under different concentrations of Cadmium Chloride were expressed as mean ± standard deviation (SD). ANOVA was used to compare parameters of untreated vs treated seedlings. P values less than 0.05 was considered to be significant.

3. Results

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

<table>
<thead>
<tr>
<th>Cd conc. (µM)</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Fresh weight (gm)</th>
<th>Dry weight (gm)</th>
<th>Protein content (gm)</th>
<th>Carbohydrate content (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.71 ±</td>
<td>11.24 ±</td>
<td>0.53 ± 0.02</td>
<td>0.09 ±</td>
<td>0.30 ± 0.02</td>
<td>0.26 ± 0.02</td>
</tr>
<tr>
<td>50</td>
<td>1.15 ± 0.43ns</td>
<td>8.22 ± 2.18**</td>
<td>0.48 ± 0.03**</td>
<td>0.08 ± 0.01ns</td>
<td>0.24 ± 0.03** (20%)</td>
<td>0.18 ± 0.03ns</td>
</tr>
<tr>
<td>100</td>
<td>1.04 ± 0.42*</td>
<td>7.48 ± 1.36**</td>
<td>0.41 ± 0.02**</td>
<td>0.07 ± 0.01**</td>
<td>0.18 ± 0.02** (40%)</td>
<td>0.15 ± 0.02*</td>
</tr>
<tr>
<td>150</td>
<td>0.88 ± 0.31*</td>
<td>7.29 ± 1.07**</td>
<td>0.39 ± 0.02**</td>
<td>0.05 ± 0.02**</td>
<td>0.14 ± 0.03**</td>
<td>0.13 ± 0.01 (50%)</td>
</tr>
<tr>
<td>200</td>
<td>0.69 ± 0.39*</td>
<td>6.84 ± 1.08**</td>
<td>0.35 ± 0.02**</td>
<td>0.03 ± 0.02**</td>
<td>0.13 ± 0.02**</td>
<td>0.09 ± 0.01* (65.38%)</td>
</tr>
<tr>
<td>250</td>
<td>0.53 ± 0.37*</td>
<td>6.12 ± 1.09**</td>
<td>0.31 ± 0.01**</td>
<td>0.03 ± 0.01**</td>
<td>0.10 ± 0.02**</td>
<td>0.05 ± 0.02* (80.77%)</td>
</tr>
</tbody>
</table>

ns = not significant, * and ** = significant at p<0.05 and p<0.01 respectively.

**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>Cd conc. (µM)</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Fresh weight (gm)</th>
<th>Dry weight (gm)</th>
<th>Protein content (gm)</th>
<th>Carbohydrate content (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.97 ± 0.38</td>
<td>12.61 ± 2.60</td>
<td>0.50 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>0.33 ± 0.02</td>
<td>0.31 ± 0.03</td>
</tr>
</tbody>
</table>
Effect of Cadmium exposures on growth and biochemical parameters of Vigna radiata seedlings

<table>
<thead>
<tr>
<th>Cd conc. (µM)</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Fresh weight(gm)</th>
<th>Dry weight (gm)</th>
<th>Protein content(gm) (gm)</th>
<th>Carbohydrate content(gm)</th>
<th>Fresh content(gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.83 ± 4.30</td>
<td>11.02 ± 0.52</td>
<td>0.52 ± 0.02</td>
<td>0.11 ± 0.03</td>
<td>0.41 ± 0.02</td>
<td>0.23 ± 0.02</td>
<td>0.3 ± 0.02</td>
</tr>
<tr>
<td>50</td>
<td>3.49 ± 0.96</td>
<td>9.73 ± 0.93</td>
<td>0.46 ± 0.01</td>
<td>0.10 ± 0.01</td>
<td>0.36 ± 0.02</td>
<td>0.16 ± 0.01</td>
<td>0.3 ± 0.02</td>
</tr>
<tr>
<td>100</td>
<td>1.86 ± 0.61</td>
<td>7.27 ± 1.27</td>
<td>0.41 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.34 ± 0.02</td>
<td>0.12 ± 0.02</td>
<td>0.3 ± 0.02</td>
</tr>
<tr>
<td>150</td>
<td>1.72 ± 0.75</td>
<td>6.97 ± 1.41</td>
<td>0.34 ± 0.03</td>
<td>0.06 ± 0.02</td>
<td>0.3 ± 0.01</td>
<td>0.08 ± 0.02</td>
<td>0.3 ± 0.02</td>
</tr>
<tr>
<td>200</td>
<td>1.19 ± 0.37</td>
<td>5.49 ± 0.65</td>
<td>0.30 ± 0.01</td>
<td>0.06 ± 0.03</td>
<td>0.24 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.3 ± 0.02</td>
</tr>
<tr>
<td>250</td>
<td>0.92 ± 0.83</td>
<td>4.97 ± 0.62</td>
<td>0.28 ± 0.02</td>
<td>0.05 ± 0.02</td>
<td>0.19 ± 0.01</td>
<td>0.03 ± 0.02</td>
<td>0.3 ± 0.02</td>
</tr>
</tbody>
</table>

ns = not significant, * and ** = significant at p<0.05 and p<0.01 respectively.

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 4: Table showing values for different parameters in untreated vs treated 7 days old seedlings in K-851 variety of V. radiata

Mamta Hirve, Angoorbala Bafna
Cadmium Chloride treated seedlings showed significantly lowered values for growth attributes (Shoot and root length, fresh and dry weights) and biochemical constituents (protein and total carbohydrate content). The root length of seedlings of Virat, SML-668, A1 Gold and K-851 varieties treated with 50 µM was insignificantly (p<0.16) decrease as compared to untreated seedlings, while those treated with 250 µM showed a significant (p<0.03) decrease. The maximum percent reduction (88.25%) was observed in A1 Gold variety (table-3). The shoot length of seedlings treated with 250 µM also shows a very significant (p<0.0001) decrease and the maximum reduction (67.82%) was observed in K-851 variety with 250 µM concentration (table-4). The fresh weight of seedlings treated with 250 µM also shows a very significant (p<0.0001) decrease as compared to untreated seedlings and the maximum reduction (66.67%) was observed in K-851 variety (table-4). The dry weight of seedlings of Virat, SML-668, A1 Gold and K-851 varieties treated with 50 µM was insignificantly (p<0.12) decreased as compared to untreated seedlings, while those treated with 250 µM shows a highly significant (p<0.0002) decrease and the maximum reduction (66.67%) was observed in Virat variety (table-1). In all varieties i.e. Virat, SML-668, A1 Gold and K-851 the total carbohydrate (CHO) content in seedlings treated with 50 µM was insignificantly (p<0.10) decrease as compared to untreated seedlings, while those treated with 250 µM showed a significant (p<0.01) decrease as compared to untreated seedlings and the maximum reduction (86.96%) was observed in A1 Gold variety at 250 µM (table-3). The protein content in 50 µM treated seedlings of Virat, SML-668, A1 Gold and K-851 was insignificantly (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).

Maximum reductions were observed in A1 Gold variety in case of 250 µM treatment of CdCl₂ i.e. 88.25% in root length and 86.96% in total sugar content as compared to untreated. All parameters of SML 668 were least affected on Cadmium Chloride treatment as compared to other varieties.

4. Discussion

4.1 Root length shoot length, fresh weight and dry weight

In the present study the root length of the seedlings was found to be decreased significantly at all concentrations (except 50 µM) of Cadmium Chloride as compared to untreated seedlings in all varieties of Vigna radiata. The shoot length of the seedlings was also found to be significantly decreased at all concentrations. These observations were in accordance with the findings of Malekzadeh P et al., (2007) who reported that root length of Zea mays was decreased in presence of Cadmium. Increasing of heavy metal concentration in root environment resulted in reduction of absorption of water and nutrients, reduction of water transpiration and disturbance in water balance, inhibition of enzymes activities, reduction of cell metabolism, reduction of photosynthesis, evaporation and transpiration, nitrogen and phosphorus shortage and stopping of growth, accelerating of maturity and even death of plant (Cheng SF., and Huang CY., 2006). Reduction in plant fresh weight under Cadmium treatment was also noted in Vigna radiata by Kumari M et al., (2011). The growth inhibition produced by Cadmium is partially due to its effect on the photosynthesis rate (Metwally A et
al, 2003). The study results showed a significant decrease in dry weight content of seedlings of V. radiata in all studied varieties. Muhammad S et al, (2008) reported that L. leucocephala seedlings showed a gradual decrease in dry weight with increase in concentration of Cadmium which was evident in the poor growth of roots and aerial parts.

4.2 Carbohydrate content

Total carbohydrate content was decreased significantly at all concentrations of Cadmium Chloride (except with 50 µM) as compared to untreated seedlings in all respective varieties of V. radiata. These results were corroborating the results of John R et al, (2008) who observed that the decrease in total carbohydrate content of stressed leaves on Cadmium treatment is probably corresponded with the photosynthetic inhibition or stimulation of respiration rate in *Lemna polyrrhiza* L. Results of Verma P et al, (2012) also shows that increasing concentration of Cadmium Chloride treatment increases the total soluble sugar content in *Vigna radiata* seedlings.

4.3 Protein content

The study result showed a significant decrease in protein content at all concentrations (except 50 µM) of Cadmium Chloride as compared to untreated seedlings in all studied varieties of Vigna radiata. These results supported the study of Verma P 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 R et al, (2008) showed that Cadmium treatment (20 mg/l) was resulted in reduction of soluble protein in *L. polyrrhiza*. Mohan BS., and Hosetti BB., (1997) found more 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 JM et al, 2002) that was found to increase under stress conditions.

5. Conclusion

Presence of Cadmium brought up changes in most of the growth parameters of V. radiata. It was concluded from the present study that there is a linear inverse relationship between the concentration of Cadmium and morphological and biochemical responses of V. radiata. Biochemical responses of plants to heavy metal concentration can view as potentially adaptive changes that decline the operation of metabolic regulatory mechanisms, which favors the functioning of the plants during or after stress. Thus, these parameters can serve as indicators of heavy metal pollution. Among all the studied varieties of V. radiata the SML 668 variety was least affected. So it is suggested that SML 668 should be grown in the areas which are polluted with Cadmium. Finally, data, which generated through this study, will be very helpful in detecting the lethal levels of heavy metals for particular plant species.

Acknowledgement

We express our sincere thanks to DR. R.K.Tugnawat Principal, Prof.R.S.Maheshwari Head Department of Biochemistry, Govt. Holkar Science College (M.P.) for providing necessary laboratory facilities and encouragement.
6. References


Effect of Cadmium exposures on growth and biochemical parameters of Vigna radiata seedlings


