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EFFECTS OF CERTAIN HEAVY METALS ON PIGMENT CONTENTS OF SEEDLINGS OF *PHASEOLUS ACONITIFOLIUS* JACQ. CV. RMO 40

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Total chlorophyll and carotenoids contents were studied in *Phaseolus aconitifolius* Jacq. cv. RMO 40 during seed germination studies. The seeds were treated with different concentrations of five heavy metals, namely, Cu, Cd, Zn, Pb and Ni. Among all the heavy metals considered, Cd was found to be the most toxic for the pigment content. The relative toxicity of heavy metals could be expressed as Cd > Pb > Ni > Cu > Zn.

Keywords : Carotenoids; Chlorophyll; Heavy metals; Seedling.

Introduction

Metals are the integral part of our planet and found in almost all rocks and soil. Men release more of the metals by burning of fossil fuel, mining, smelting and discharging industrial, agricultural and domestic wastes. The metallic elements are often divided into light metal and heavy metal. The term heavy metal refers to the metal with potential toxicity, having density greater than 4.0 grams per cubic cm^1 and it is a metal which can be precipitated in acid solution by hydrogen sulphide. Heavy metals have significant biological roles as metallo-enzyme and are required as micronutrients by all organisms[2]. However, they prove to be hazardous when the concentration of these metals is slightly increased above the quantity in traces needed for nutritional requirements and physiological interaction.

Phaseolus aconitifolius (moth bean) is an important arid legume crop grown extensively in arid and semi arid parts of the country. It is a highly drought tolerant crop cultivated with a minimum of inputs and is the best insurance for marginal land in case of crop failure due to drought. It is an important food legume for sustainable economic importance in hot desert regions of the country. The seeds are rich in protein (22-26%) and make an excellent supplement to cereal diets. The seeds are used either whole or as a pulse. Moth bean is a good source of amino acids particularly lysine, leucine and certain vitamin, like carotene.

Among the phytochemical estimations, photosynthetic pigments are the most important parameters. Chlorophyll content gives a good idea about the productivity of plants. There is a close correlation between the amount of chlorophyll and the rate of photosynthesis, which may vary or fluctuate with response to application of minerals elements including pollutants or heavy metals.

Material and Methods

Certified seeds of *Phaseolus aconitifolius* Jacq. cv. RMO 40 were obtained from Research Station Beechwal, Rajasthan Agriculture University, Bikaner. Seeds were stored in glass stoppered bottles. After a preliminary selection for uniformity criteria (size and colour of seeds), the seeds were surface sterilized with 0.1% $HgCl_2$ for two minutes[3], then washed with distilled water three times and then soaked for two hours in respective solutions of different concentrations (10, 50, 100, 200, 500 and 1000 ppm) of copper sulphate, cadmium sulphate, lead sulphate, nickel sulphate and zinc sulphate. Seeds soaked in distilled water for two hours served as the ~~control. After~~ the above treatments, seeds were removed and allowed to germinate in petri plates on filter paper soaked in each of the above metallic solution. Three replicates each of 10 seeds were kept for each concentration of every heavy metal. The filter paper was moistened with metallic solutions. The experiments were carried out for ten days under laboratory conditions of temperature (25±2°C) and diffuse light. On the day of termination of experiment, (10th day) germinated seeds were counted, total chlorophyll and carotenoid contents were recorded from the germinated seeds (seedlings). Total chlorophyll and total carotenoids were determined by the method of Arnon[4] and Kirk and Allen[5], respectively.

Results and Discussion

The data regarding the effect of heavy metals on total chlorophyll and carotenoid contents are recorded in Tables 1 and 2.

Table 1. Showing the effect of heavy metals on total Chlorophyll contents (mg/g fresh weight) in the seedling of *Phaseolus aconitifolius* cv. RMO 40.

Heavy metals	Concentrations (ppm)						
	Control± SD	10 ± SD	50± SD	100± SD	200± SD	500± SD	1000± SD
Cu	2.227±0.006	3.010±0.010	3.740±0.020	4.220±0.020	3.440±0.020	2.260±0.00	1.170±0.010
Cd	2.227±0.006	1.607±0.284	1.557±0.015	1.373±0.012	1.263±0.015	0.470±0.010	0.000±0.000
Zn	2.227±0.006	3.060±0.010	4.063±0.015	4.493±0.059	3.423±0.021	2.213±0.015	2.140±0.014
Pb	2.227±0.006	3.470±0.010	4.150±0.026	3.450±0.010	3.043±0.010	2.047±0.006	2.007±0.012
Ni	2.227±0.006	2.723±0.015	3.227±0.015	3.420±0.020	3.167±0.031	2.123±0.015	2.150±0.010

Table 2. Showing the effect of heavy metals on Carotenoid contents (mg/g fresh weight) in the seedling of *Phaseolus aconitifolius* cv. RMO 40.

Heavy metals	Concentrations (ppm)						
	Control± SD	10± SD	50± SD	100± SD	200± SD	500± SD	1000± SD
Cu	0.473±0.012	0.50±0.036	0.523±0.040	0.553±0.042	0.533±0.045	0.440±0.017	0.397±0.058
Cd	0.473±0.012	0.443±0.095	0.290±0.052	0.277±0.060	0.270±0.050	0.263±0.015	0.000±0.000
Zn	0.473±0.012	0.600±0.020	0.647±0.031	0.710±0.053	0.657±0.080	0.447±0.006	0.400±0.010
Pb	0.473±0.012	0.373±0.153	0.313±0.095	0.363±0.015	0.390±0.040	0.373±0.012	0.327±0.100
Ni	0.473±0.012	0.473±0.040	0.507±0.064	0.547±0.081	0.487±0.040	0.457±0.031	0.453±0.059

SD = Standard Deviation

(i) *Effect of Heavy Metals on Total Chlorophyll Content* : In comparison to the control, there was gradual increase in chlorophyll contents at lower concentrations *i.e.* 10 ppm to 100 ppm of heavy metals (Zn, Cu, Pb and Ni).In Cd it decreased even at lowest concentration *i.e.* 10 ppm (Table 1). At higher concentrations *i.e.* at 1000 ppm, chlorophyll content was 1.17 mg/g in Cu, 2.14 mg/g in Zn,2.15 mg/g in Ni and 2.00 mg/g in Pb whereas in the control it was 2.22 mg/g. Seedling did not survive at 1000 ppm concentration of Cd only.

(ii) *Effect of Heavy Metals on Carotenoid Content* : Carotenoid content was not very much affected by Zn, Cu and Ni in comparison to Pb and Cd. Carotenoid content increased up to 200 ppm of Zn, Cu and Ni and then gradually decreased with further increase in heavy metal concentrations (Table 2). Gradual decrease was observed in Cd with increase in concentration upto 1000 ppm. Carotenoid content was 0.39 mg/g in Cu, 0.40 mg/g in Zn, 0.45 mg/g in Ni and 0.32 mg/g in Pb at 1000 ppm concentration as compared to control where it was 0.47 mg/g. Sharp decline was observed at different concentrations of Cd. Seedling died at 1000 ppm concentration.

A perusal of the observations (Table 1 and 2) on pigment contents reveal that both chlorophyll and carotenoid contents decreased significantly in *Phaseolus aconitifolius* cv. RMO 40 with the application of heavy metals. In general, all the heavy metals were lowered the pigment contents except certain lower concenteration of Cu, Zn, Pb and Ni. The decrease in pigment content in *Phaseolus aconitifolius* might have been due to inhibition of several physiological activities mainly photosynthesis. It may be due to alteration in synthesis of structural component which in turn affect metabolism of plant. A perusal of the literature reveal that different explanation have been put forth for the reduction in pigment content by the application of heavy metal.

Siedlecka *et al.*[6] reported reduced accumulation of chlorophyll in Cd, Cu, Hg, Pb, Mn and Ni treated angiosperm plants. Moreover, different heavy metals are

able to substitute Mg as a central ion in the porphyrin ring of chlorophyll or bacteriochlorophyll[7-8]. Cicek and Cakirlar[9] observed that salt stress caused a decline in plant height and biomass, total chlorophyll content and ion imbalances and an increase in the solute leakage, proline content exhibited variations dependent on cultivars and temperatures and these variations were reflected in Chl a fluorescence parameters. Alteration in photosynthetic performance of *Salvinia natans* (L.) exposed to chromium, zinc rich waste water were investigated by Dhir *et al.*[10]. They observed that accumulation of high levels of Cr and Zn affected photosynthetic electron transport in plants.

Gautam *et al.*[11] observed that addition of Cd in the growth medium also had significant deleterious effect on net photosynthetic rate. Chlorophyll and carotenoid content remained almost unaffected at lower concentration of Pb tested in *Vigna radiata*. Dube *et al.*[12] reported that excess of Cd (40 mg/kg soil) showed significant decreased in concentration of chlorophyll 'a' and 'b' in spinach leaves.

Change in photosynthetic pigments and photosynthetic electron transport activity in a liverwort *Riccia* spp. was also reported by Prasad *et al.*[13] in favour of present study. Symeonidis and Karataglis[14] observed negative correlation between chlorophyll content and increased heavy metal concentrations in *Holchus lanalus* L. The greater chlorophyll content found in tolerant genotype, in different Pb and Zn concentration, in comparison with the control, could be served to distinguish tolerant and non-tolerant genotype. Zhang and Huang[15] performed a pot culture experiment to study the effect of Cd stress on leaf chlorophyll content. They concluded that Cd reduced chlorophyll content and changed the chlorophyll a/b ratio. Effect of heavy metals on pigment contents of seedling of *Cyamopsis tetragonoloba* cv. RGC 936 and cv. RGC 1002 was studied by Jain and Bhansali[16,17]. It was observed that Cd and Pb in comparison to Zn, Cu and Ni drastically reduced the total chlorophyll content at 1000 ppm concentration.

Decreased chlorophyll, carotenoid concentration and increase in chlorophyll a/b ratio and de-epoxidation of violaxanthin cycle pigments in presence of zinc sulphate was observed by Sagardoy *et al.*[18] in sugar beet. Higher concentrations of Zn decrease photosystem II efficiency. The interactive functions of Zn and Cd on photosynthetic apparatus in *Ceratophyllum demersum* were investigated by Aravind and Prasad[19]. They concluded that Cd was not only exhibited pronounced toxicity on the over all photosynthetic machinery but also on the pigment biosynthesis.

Among all the heavy metals considered, Cd was found to be the most toxic for the pigment contents of *Phaseolus aconitifolius* cv. RMO 40. The relative toxicity of heavy metals could be expressed as Cd>Pb>Ni>Cu>Zn.

References

1. Krishnamurti C R 1985, Environment, chemicals and man. *Madras Science Foundation* 138.
2. Wood J, Segall H J, Bideley W P, Chen A, Chudyk W and Thayer J S 1975, Proc. Inter. Conf. on heavy metals in the environment. Toronto, Ontario, Canada. 49 68.
3. Misra R 1968, *Ecology Work Book*. Oxford and IBH Publishing Co. New Delhi, Bombay, Calcutta.
4. Arnon D I 1949, Copper enzyme in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* **24**(1) 1-15.
5. Kirk J T O and Allen R L 1965, Dependence of chloroplast pigment synthesis on their protein synthesis. Effect of actidione. *Biochem. Res. Common.* **21** 523-530.
6. Siedlecka A, Tukiendorf A, Skorzynska, Polit E, Maksymiec M, Bazynski T and Krupa Z 2001, Selected angiosperm families response to heavy metals on the Environnment. In : *Metals in the Environment : Analysis by Biodiversity*, Prasad, MNV, (Ed.), Marcel Dekker Inc. 171-218.
7. Puckett K J 1976, The effect of heavy metals on some aspects of lichen physiology. *Can. J. Bot.* **54** 2695-2703.
8. Kupper H, Kupper F and Miller S 1996, Environmental relevance of heavy metal substituted chlorophyll using the example of water plants. *J. Exptl. Bot.* **47** 259-266.
9. Cicek N and Cakirlar H 2008, Effect of salt stress on some physiological and photosynthetic parameters at three different temperatures in six soyabean (*Glycine max* L. Merr.) cultivars. *J. Agron. Crop. Sci.* 194 34-46.
10. Dhir B, Sharmila P and Pardha Saradhi P 2008, Photosynthetic performance of *Salvinia natans* exposed to chromium and zinc rich wastewater. *Braz. J. Pt. Physiol.* **20** 61-70.
11. Gautam M, Senger R S, Garg S K, Senger K and Choudhary R 2008, Effect of lead on seed germination, seedling growth, chlorophyll content and nitrate reductase activity in mungbean (*Vigna radiata*). *Res. J. Phytochem.* **2** 61-68.
12. Dube B K, Sinha P and Chatterjee C 2002, Changes in spinach (*Spinacea oleracea* L.) metabolites by excess cadmium. *Nat. Environ. Polln. Techno.* **1** 225-229.

13. Prasad S M, Dwivedi R, Zeesham M and Singh R 2004, UV-B and Cd induced changes in pigments, photosynthetic electron transport activity antioxidant levels and antioxidative enzymes activity of *Riccia sp. Acta Physiol. Plant.* **26** 423-430.
14. Symeonidis L and Karataglis S 2008, The effect of lead and zinc on plant growth and chlorophyll content of *Holchus lanalus* L. *J. Agron. Crop. Sci.* **168** 108-112.
15. Zhang J B and Huang W N 2007, Effect of cadmium stress on photosynthetic functions of strawberry. *Ying Yong Sheng Tai Xue Bao.* **18** 1673-1676.
16. Jain U and Bhansali S 2008a, Effect of certain heavy metals on pigment contents of seedling of *Cyamopsis tetragonoloba (L.) Taub. cv. RGC 936. J. Phytol. Res.* **21** 127-130.
17. Jain U and Bhansali S 2008b, Effect of certain heavy metals on pigment contents of seedlings of *Cyamoposis tetragonoloba* (L.) Taub. cv. RGC 1002. *Ind. J. Environ. Sci.* **12** 129-132.
18. Sagardoy R, Morales F, Lobez-Millan A F, Abadia A and Abadia J 2008, Effect of zinc toxicity on sugarbeet (*Beta vulgaris* L.) plants grown in hydroponics. *Pt. Biol. (German Society) Royal Botanical Society of Netherlands* ISSN 1435-8603.
19. Aravind P and Prasad M N V 2004, Zinc protects chloroplasts and associated photochemical functions in cadmium exposed *Ceratophyllum demersum* L., a fresh water macrophyte. *Pt. Sci.* **166** 1321-1327.