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EFFECT OF CERTAIN HEAVY METALS ON PIGMENT CONTENTS OF SEEDLINGS OF *CYAMOPSIS TETRAGONOLOBA (L.)* TAUB. CV. RGC 936

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Cyamopsis tetragonoloba (L.) Taub. is known as "guar". Total chlorophylls and carotenoids were calculated from seedlings treated with five heavy metals. It was observed that Cd and Pb in comparison to Zn, Cu and Ni drastically reduced the total chlorophyll content at 1000 ppm concentration. The relative toxicity of heavy metals could be expressed as Cd> Pb> Ni> Zn> Cu.

Keywords: Carotenoids; Chlorophyll; Heavy metals; Seedlings.

Introduction

Heavy metals can be defined as one that has a density greater than 4.0 g per cubic centimeter[1]. Some heavy metals at low doses are essential micronutrients for plants but in higher doses they cause metabolic disorders and growth inhibition for most of plant species[2].

Cyamopsis tetragonoloba (L.) Taub. is an important green fodder plant. Due to multiple importance and diversified uses as feed, fodder, vegetable and industrial seed gum, guar now has come to occupy the status of commercial crop in India during the last two decades.

Various pigments are found in plants out of which chlorophylls are the most important. The chlorophyll pigments are used in photosynthesis, to some extent in medicines, as a colouring agent for waxes, candles, resins, soaps, food, oil and as deodorants. Both chlorophyll 'a' and chlorophyll 'b' show an absorption maximum in the blue violet region with peaks of about 429 nm and 453 nm, respectively and with minor peaks at 410 nm and 430 nm. They also have a secondary absorption which is maximum in the red region with peaks of about 660 nm and 642 nm.

Besides chlorophylls, there are yellowish coloured pigments called carotenoids which always remain in association with chlorophylls. No light is required for their formation. The absorption peaks of carotenoids lie between 425 nm and 490 nm. They prevent photo-oxidation of chlorophylls. Photosynthetic pigments are associated with the internal membranous lamellae or thylakoids of chloroplasts. Among the biochemical estimations, photosynthetic pigments are the most important parameters. Chlorophyll content gives good idea about the productivity of plants and is an index of community function. There is a close correlation between the amount of chlorophyll and the rate of photosynthesis. The relationship remains constant for different species of plants and may vary with the application of mineral elements including the heavy metals.

Materials and Methods

Certified seeds of *Cyamopsis tetragonoloba* L. Taub. cv. RGC 936 were obtained from Durgapura Agriculture Research Station, Jaipur. 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 nitrate, nickel sulphate and zinc sulphate. Seeds soaked in distilled water for two hours constituted 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 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 germinated seeds were counted, total chlorophyll and carotenoids were recorded.

Chlorophyll 'a+b' (total chlorophyll) and total carotenoids were determined by the method of Arnon[4] and Kirk and Allen[5], respectively.

Result and Discussion

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

(i) Effect of Heavy Metals on Total Chlorophyll Content: With increase in concentrations of heavy metals there was a gradual decrease in total chlorophyll content in

Table 1. Showing the effect of heavy metals on total chlorophyll content (mg/g fresh weight) in seedlings of *Cyamopsis tetragonoloba* cv. RGC 936 (values are means of three replicates each).

Name of chemical	Control	Concentration (ppm)					
		10	50	100	200	500	1000
Cadmium sulphate	0.97	0.85	0.84	0.82	0.77	0.72	0.67
Lead nitrate	0.97	0.92	0.89	0.87	0.83	0.76	0.70
Nickel sulphate	0.97	0.98	0.92	0.89	0.86	0.81	0.74
Zinc sulphate	0.97	0.99	0.94	0.91	0.88	0.84	0.85
Copper sulphate	0.97	1.2	1.2	0.98	0.97	0.86	0.79

Source	D.F.	S.S.	M.S.S.	'F' ratio
Replication	2	0.029	0.0145	3.15^{NS}
Control vs Treatment	1	0.15	0.15	32.60***
Among Concentrations	6	0.51	0.085	18.47***
Among chemicals	4	0.34	0.085	0.20^{NS}
Interaction	2	1.46	0.7325	18.47***
Error	89	-0.41	0.0046	-

Table 2. Showing the effect of heavy metals on Carotenoid content (mg/g fresh weight) in the seedlings of *Cyamopsis tetragonoloba* cv. RGC 936 (values are means of three replicates each).

Name of chemical	Control	Concentration (ppm)					
		10	50	100	200	500	1000
Cadmium sulphate	0.37	0.31	0.29	0.28	0.25	0.21	0.11
Lead nitrate	0.37	0.33	0.30	0.30	0.28	0.25	0.20
Nickel sulphate	0.37	0.38	0.33	0.30	0.28	0.25	0.23
Zinc sulphate	0.37	0.39	0.33	0.33	0.30	0.29	0.26
Copper sulphate	0.37	0.38	0.35	0.34	0.30	0.27	0.26

Source	D.F.	S.S.	M.S.S.	'F' ratio
Replication	2	0.002	0.001	0.416^{NS}
Control vs Treatment	1	0.08	0.08	33.3***
Among Concentrations	6	0.20	0.03	2.5***
Among chemicals	4	0.02	0.005	2.083^{NS}
Interaction	2	0.094	0.047	19.58***
Error	89	0.22	0.0024	-

NS = Not significant

*** = Highly significant

Cyamopsis tetragonoloba cv. RGC 936 except in respect of Zn and Cu up to 10 and 50 ppm concentration, respectively, where it was superior to control. Highly significant results were recorded for control *versus* treatments and among various concentrations themselves. No such differences were obtained among various chemicals and among replicates. It was observed that Cd and Pb in comparison to Zn, Cu and Ni drastically reduced the total chlorophyll content at 1000 ppm concentration.

(ii) Effect of Heavy Metals on Carotenoid Content : It was observed that Zn and Cu were less inhibitory to the amount of total carotenoid content in comparison to other heavy metals. In control, the carotenoid content was 0.37 mg/g fresh weight which decreased to 0.11 mg/g in Cd, 0.20 mg/g in Pb, 0.23 mg/g in Ni, 0.26 mg/g in Zn and Cu of fresh weight at 1000 ppm concentration (Table 2). At 1000 ppm conentration, Cd and Pb caused the highest reduction in pigment content. Statistically highly significant results were observed between control *versus* treatment and among various concentrations, whereas non significant results were noticed among various chemicals and replicates.

A perusal of the observations (Table 1 and 2) on pigment content reveals that both total chlorophyll and carotenoid contents decreased significantly in *C. tetragonoloba* with the application of heavy metals. In general, all the concentrations of heavy metals were inhibitory to the pigment contents in *C. tetragonoloba* except 10 ppm concentration of Ni, Zn and Cu for the chlorophyll content. A perusal of the literature reveal that different explanations have been put forth for the reduction in pigment content by the application of heavy metals. Bohner *et al.*[6] reported that Cu inhibited chlorophyll concentration which might be due to inhibited photosynthetic electron transport. Dube *et al.*[7] reported that excess of Cd (40 mg/kg soil) significantly decreased concentration of chlorophyll 'a' and 'b' in spinach leaves. Oza and Kumar[8] reported that with increase in Cd levels, the concentration of chlorophyll decreases in spinach leaves. Heavy metal treatments caused oxidative stress in plants leading to reductions in photosynthetic pigments and consequently the biomass of spinach plants[9]. On the contrary, Pratima[10] reported that plant irrigated with treated and enriched effluents showed greater shoot, total length and higher chlorophyll-a and -b content and total chlorophyll content, indicating the importance of treatment and enrichment of effluents. Muthuchelian *et al.*[11] investigated an increase in the chlorophyll synthesis of *Phaseolus aureus* grown in sewage soil. Prasad *et al.*[12] reported changes due to Cd o photosynthetic pigments and photosynthetic electron transport activity in a liverwort, *Riccia* species. The present findings on the inhibition of the pigment content in *C. tetragonoloba* in the presence of heavy metals are in agreement with the earlier reports where low values of pigment contents were obtained under treatment of lead in *Vigna mungo, Zea mays* cv. Co. 1[13-14], under cadmium in *Triticum aestivum*[15], under nickel in chickpea[16], under copper in lentil[17] and under zinc in spinach chloroplast[18]. Keshav and Mukherji[19] and Kalita *et al.*[15] concluded that inhibition of chlorophyll content in mung bean *(Vigna radiata)* in the presence of Cd may be due to the interference with the synthesis of protein which is the structural component of chloroplasts. Kumar[20] reported that in *Catharanthus roseus* the leaf senescence was accelerated by higher dose of heavy metals and Cd caused the highest toxic effect. Among all the heavy metals considered, Cd was found to be the most toxic for the pigment contents of *Cyamopsis tetragonoloba* cv. RGC 936. The relative toxicity of heavy metals could be expressed as Cd> Pb> Ni> Zn> Cu.

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