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EFFECT OF CERTAIN HEAVY METALS ON PIGMENT CONTENTS OF SEEDLINGS OF *DAUCUS CAROTA* L.

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Total chlorophyll and carotenoids contents in seedlings of *Daucus carota* L. decreased when they were treated with five heavy metals namely, cadmium, lead, nickel, zinc and copper. When compared with controls Cd was found to be the most toxic to pigments. The relative toxicity of heavy metals could be expressed as Cd>Pb>Ni>Cu>Zn.

Keywords : Carotenoids; Chlorophyll; Heavy metals; Seedlings.

Introduction

The term heavy metal refers to the metal with potential toxicity, having density greater than 4.0 gram per cubic cm[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]. *Daucus carota* L. commonly known as wild carrot, garden carrot is a flowering plant of the family Apiaceae. Plant pigments are the synthetic products of plants formed during metabolic activities of cell. Chlorophyll is the most important pigment in plants. They are essential for photosynthesis and represent the green colour of plants. In the process of photosynthesis, carbohydrates, fats and proteins are synthesized by absorbing light energy in the presence of chlorophyll. Because of this food synthesizing character, the chlorophyll pigments are regarded among the most important chemical substances in nature.

Besides chlorophylls, there are yellowish colured pigments called carotenoids which always remain in association with chlorophylls. They cannot transfer sunlight energy directly to the photosynthetic pathway, but pass their absorbed energy to chlorophyll. 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.

Material and Methods

Certified seeds of *Daucus carota* L. were obtained from local market, Jaipur. Seeds were stored in glass stoppered bottles. After a preliminary selection for uniformity criteria (size and colour), 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 1000ppm) of copper sulphate, cadmium sulphate, lead nitrate, nickel sulphate and zinc sulphate. Seeds soaked in distilled water for two hours constituted the conrol. After the above treatment, seeds were removed and allowed to germinate in Pertri plates on filter paper soaked in each of the above metallic solutiion. Three replicates each of 10 seeds were kept for each concentration of every heavy metal. The filter paper was moistened with metallic solution. 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. The data were statistically analysed and F-ratio calculated[6-7].

Result and Discussion

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

(i). *Effect of heavy metals on total chlorophyll content :* It was observed that all the heavy metals were inhibitory to total chlorophyll contents of *Daucus carota* L. (Table 1). The cadmium showed marked reduction at 200 ppm onward. Total chlorophyll content in control was 0.87 mg/g fresh weight. It decreased to nd (not detectable) in 1000 ppm concentration of all the heavy metals and 500 ppm concentration of Cd. It decreased to 0.50 mg/g fresh weight in Pb, 0.55 mg/g fresh weight in Ni, 0.57 mg/g

fresh weight of Cu and 0.58 mg/g fresh weight in Zn at 500 ppm concentration when compared with control. Statistically, results were found to be highly significant between control *versus* treatments and significant among various concentrations. The results were found to be not significant among five heavy metals.

(ii) *Effect of heavy metals on carotenoid content*- It was observed that Ni and Zn were less inhibitory to the amount of total carotenoid content in comparision to other heavy metals. In c

ontrol the carotenoid content was 0.33 mg/g fresh weight which decreased to nd(not detectable) in Cd, 0.11 mg/g in Pb, 0.16 mg/g in Cu, 0.18 mg/g in Zn and 0.14 mg/g in Ni of fresh weight at 500 ppm concentration (Table 2). At 1000 ppm concentration, Cd and Pb caused the highest reduction in pigment content. Statistically highly significant results were observed between control *versus* treatment and replicates, whereas non significant results were noticed among various chemicals and among various concentrations.

A perusal of the observations (Table 1 and 2) on pigment contents reveal that both total chlorophyll and carotenoid contents decreased significantly in *Daucus carota* with the application of heavy metals. In general, all the concentrations of heavy metals were inhibitory to the pigment contents in *D. carota* except 10 ppm concentreation of Ni, Zn and Cu for the chlorophyll and carotenoid contents. A perusal of the literature reveals that different explanations have been put forth for the reduction in pigment content by the application of heavy metals. Bohner *et al.*[8] reported that Cu inhibited chlorophyll concentation which might be due to inhibited photosynthetic electron transport. Dube *et al.*[9] observed that excess of Cd (40 mg/kg in soil) significantly decreased concentration of chlorophyll 'a' and 'b' in spinach leaves. Oza and Kumar[10] had opinion that with increase in Cd levels, the concentration of chlophyll decreases in spinach leaves. A characteristic feature of toxicities due to heavy metals is chlorosis and reduction in the net photosynthetic rate leading to decreased growth and productivity[11]. Among heavy metal pollutants, nickel (Ni) and cadmium (Cd) need special attention due to their widespread occurrence and potential for their toxicities. Cd in higher concentration has been shown to disturb chlorophyll synthesis[12] and many metabolic processes like nucleic acid and protein synthesis[13]. Ni is cosidered as an essential micronutrient for plants but is strongly phytotoxic at higher concentrations[14]. Ni-induced deactivation of proteins including antioxidant enzymes, lipid peroxidation and membrane function have been reported in plants[15]. Synergistic action of UV-B radiation and Cd was reported by Shukla *et al.*[16] on the growth of wheat seedlings. Prasad *et al.*[17] reported changes due to Cd on photosynthetic pigments and photosynthetic electron transport activity in a liverwort, *Riccia* species. Reduction in chlorophyll contents by excess Ni and Cd has been reported in the pigeon pea[18]. Carotenoids protect chlorophyll from photooxidative destruction[19] and therefore, a reduction in carotenoid could have a serious consequence on chlorophyll pigments. 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[20,21]. It was observed that Cd and Pb in comparison to Zn, Cu and Ni drastically reduced the total chlorophyll content at 1000 ppm concentration. Effect of heavy metals on pigment contents of seedling of *Brassica rapa* L.was also studied by Jan *et al.*[22]. Keshav and Mukherji[23] and Kalita *et al.*[24] 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 chloroplast. Kumar[25] 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 metal considered, Cd was found to be the most toxic for the pigment contents of *Daucus carota* L. The relative toxicity of heavy metals could be expressed as Cd>Pb>Ni>Cu>Zn.

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Table 1. Showing the effect of heavy metals on total chlorophyll contents (mg/g fresh weight) in seedling of *Daucus carota* L. (values are means of three replicates each).

Name of chemicals	Cont.	Concentration (ppm)					
		10	50	100	200	500	1000
Copper Sulphate	0.87	0.82	0.80	0.75	0.68	0.57	nd
Cadmium Chloride	0.87	0.80	0.77	0.70	0.59	nd	nd
Lead Nitrate	0.87	0.81	0.79	0.72	0.61	0.50	nd
Nickel Sulphate	0.87	0.80	0.78	0.75	0.66	0.55	nd
Zinc Chloride	0.87	0.86	0.83	0.79	0.69	0.58	nd

Source	D.F.	S. S.	M.S.S.	'F' ratio
Replication	2	0.0056	0.0134	1.03^{NS}
Control vs Treatment	1	0.18	0.19	36.50**
Among concentrations	6	0.68	0.065	18.07*
Among chemicals	4	0.98	0.076	0.90^{NS}
Interaction	2	1.77	0.778	15.23*
Error	89	0.78	0.0023	–

Table 2. Showing the effect of heavy metals on carotenoid contents (mg/g fresh weight) in the seedling of *Daucus carota* L. (values are means of three replicates each).

Name of chemicals	Cont.	Concentration (ppm)					
		10	50	100	200	500	1000
Copper Sulphate	0.33	0.31	0.29	0.26	0.20	0.16	nd
Cadmium Chloride	0.33	0.27	0.20	0.18	0.12	nd	nd
Lead Nitrate	0.33	0.27	0.22	0.20	0.14	0.11	nd
Nickel Sulphate	0.33	0.28	0.25	0.23	0.17	0.14	nd
Zinc Chloride	0.33	0.35	0.30	0.29	0.21	0.18	nd

Source	D.F.	S. S.	M.S.S.	'F' ratio
Replication	2	0.053	0.033	0.896^{NS}
Control vs Treatment	1	0.009	0.076	28.3**
Among concentrations	6	0.33	0.065	10.5^{NS}
Among chemicals	4	0.67	0.0022	-1.034^{NS}
Interaction	2	0.094	0.066	12.09*
Error	89	0.34	0.038	–

*=significant; **=Highly significant; NS=Not significant

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