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EFFECT OF CERTAIN HEAVY METALS ON PIGMENT CONTENTS OF SEEDLINGS OF *BRASSICA RAPA* L.

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Total chlorophyll and carotenoids contents in seedlings of *Brassica rapa* L. decreased when 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 > Cu > Zn > Ni.

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].

Brassica rapa L. commonly known as field mustard or turnip mustard is a plant widely cultivated as a leaf vegetable, a root vegetable and an oil seed. Plant pigments are the synthetic products of plants formed during metabolic activities of cell. Chlorophyll is the most important pigment in plants. Because of food synthesizing character, the chlorophyll pigments are regarded among the most important chemical substances in nature.There are several kinds of chlorophylls, the most important being chlorophyll 'a'. and chlorophyll 'b'. Besides chlorophylls, there are yellowish coloured pigments called carotenoids which always remain in association with chlorophylls. They prevent photo-oxidation of chlorophylls. 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 *Brassica rapa* L. 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 each 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 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 germinated seeds were counted, total chlorophyll and carotenoids were estimated.

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].

Results 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 seedlings of *Brassica rapa* L. except Ni and Zn up to 10 and 50 ppm concentration, in comparison to other heavy metals like Cd, Pb and Cu. Highly significant results were recorded for among replicates and among various concentrations themselves. No such differences were obtained among various chemicals and control *versus* treatments. It was observed that Cd and Pb in comparison to Ni, Zn and Cu drastically reduced the total chlorophyll

Table 1. Effects of heavy metals on chlorophyll content (mg/g fresh weight) of *Brassica rapa* L. seedlings (values are means of three replicates each).

Treatment	Control	Concentration (ppm)					
		10	50	100	200	500	1000
Cadmium sulphate	0.90	0.67	0.54	0.50	0.47	0.40	-
Lead nitrate	0.90	0.69	0.57	0.49	0.51	0.50	-
Nickel sulphate	0.90	0.87	0.80	0.67	0.69	0.65	0.49
Zinc sulphate	0.90	0.76	0.70	0.61	0.59	0.51	0.48
Copper sulphate	0.90	0.69	0.66	0.60	0.52	0.50	0.40

Source	D.F.	S.S.	M.S.S.	'F' ratio
Replication	2	0.0184	0.0092	0.5907341***
Control *vs* Treatment	1	0.21	0.21	0.0620271^{NS}
Among concentrations	6	0.24	0.04	0.0118147***
Among chemicals	4	0.07	0.0175	0.0051689^{NS}
Interaction	2	0.745	0.3725	0.1100242***
Error	89	301.32	3.385	1

NS = Not significant
*** = Highly significant

Table 2. Showing the effect of heavy metals on carotenoid content (mg/g fresh weight) in the seedlings of *Brassica rapa* L.

Treatment	Control	Concentration (ppm)					
		10	50	100	200	500	1000
Cadmium sulphate	0.41	0.28	0.26	0.21	0.19	0.15	0.10
Lead nitrate	0.41	0.30	0.28	0.23	0.20	0.17	0.13
Nickel sulphate	0.41	0.42	0.38	0.35	0.30	0.29	0.25
Zinc sulphate	0.41	0.42	0.37	0.30	0.28	0.27	0.22
Copper sulphate	0.41	0.37	0.32	0.25	0.24	0.20	0.18

Source	D.F.	S.S.	M.S.S.	'F' ratio
Replication	2	0.00125	0.000625	0.1386628***
Control *vs* Treatment	1	1.56	1.56	0.108157***
Among concentrations	6	1.88	0.31333	0.0217238^{NS}
Among chemicals	4	0.83	0.2075	0.0143863^{NS}
Interaction	2	4.664	2.332	0.1616808***
Error	89	1283.69	14.423	1

NS = Not significant
*** = Highly significant

content at 500 and 1000 ppm concentrations.

(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 comparison to other heavy metals. In control the carotenoid content was 0.41 mg/g fresh weight which decreased 0.10 mg/g in Cd, 0.13 mg/g in Pb, 0.18 mg/g in Cu, 0.22 mg/g in Zn and 0.25 mg/g in Ni of fresh weight at 1000 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 revealed that both total chlorophyll and carotenoid contents decreased significantly in *Brassica rapa* with the application of heavy metals. In general, all the concentrations of heavy metals were inhibitory to the pigment contents in *Brassica rapa* except 10 ppm concentration of Ni, Zn and Cu for the chlorophyll and carotenoid contents. A perusal of the literature reveal revealed 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 concentration 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 chlorophyll 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 considered 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 pigments 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. Keshav and Mukherji[22] and Kalita *et al.*[23] 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[24] 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 *Brassica rapa* L. The relative toxicity of heavy metals could be expressed as Cd > Pb > Cu > Zn > Ni.

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