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Chromium (Cr) phyto-toxicity effect of Horse gram (*Dolichos biflorus* L.)

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Abstract

In recent years, Heavy Metals (HMs) pollution has become a serious problem all over the world as these (HMs) continue in the soil for longer period due to their non-biodegradability. The chromium (Cr) is one of the nonessential heavy metal that makes highly toxicity in the plants as well as soil. Cr is one such nutrient required for sugar and fat metabolism in humans, whereas the role of Cr in plant growth and its uptake pathway are not yet fully understood. The pot experiments were carried out of the Horse gram (*Dolichos biflorus* L.) seeds were raised in pots, the pots containing 2.5 kg of soil with adding different levels of Cr (control, 2, 4, 6, 8 and 10 mg/kg soil). Three replicates were maintained for each level. Morphological parameters like root and shoot length, total leaf area and fresh and dry weights and pigment content and biochemical estimation were recorded in 60th DAS of plants. From the results was concluded that the 2 mg kg^{-1} level of Cr in the soil was beneficial for the growth of Horse gram. The level of Cr in the soil above 2 mg kg^{-1} proved to be toxic effects. The results indicated that the 2 mg kg^{-1} Cr level can be increasing the growth of Horse gram.

Keywords: *Chromium, Toxicity, Dolichos biflorus L., Pigments and Biochemical content*

1. Introduction

Environmental pollution with Heavy Metals (HMs) is a worldwide danger that is related to human activities such as mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping and melting operation. The pollution has many forms, the air we take breaths, the water we drink, the ground where we cultivated our food crops and even the increasing noise we hear everyday—all contribute to health problems and minor eminence of life (Datta *et al*., 2011). The HMs pollution mainly arises from the effluents of industrial units, some of the industrial units releasing toxic HMs into environment. The effluents released from paper mills and fertilizer factories are adding a mixture of alkali, ammonia, cyanides and HMs into the water resources. The waste water from the dyes and pigment industries, film and photography, galvanometry, metal cleaning, electroplating, leather and mining industries contains considerable amounts of HMs (Cervantes *et al*., 2001).

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Among the HMs pollutants is most dangerous one as these are non-biodegradable and continue in environment. These come into the water resources through both natural and anthropogenic resources. More attention is being given to the potential health hazards posed by HMs. The term HMs refers to any metallic chemical element that has a relatively high density (Pillay *et al.*, 2003). The density of HMs is usually more than 5.0 g/cm^3. Examples of HMs include Mercury (Hg), Cadmium (Cd), Arsenic (As), Chromium (Cr), Thallium (Tl), Lead (Pb), Copper (Cu), Zinc (Zn), Cobalt (Co), Nickel (Ni), and Iron (Fe). These metals are classified in to three categories, toxic metals (such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc.), precious metals (such as Pd, Pt, Ag, Au, Ru, etc.) and radionuclides (such as U, Th, Ra, Am, etc.) (Dube *et al.*, 2003). The metals cause toxicity to organisms even at ppm level of concentration.

Cr is recognized as non-essential plant nutrient however trace amounts of chromium is required for living organisms (Subha Priya and Rajeshwari 2013). But in few studies it has been reported that traces amount of Cr may increase plant productivity (Orhue and Ekhomun, 2010; Manivasagaperumal *et al.*, 2011; Srivastava and Shukla 2016; and Pandey and Pandey 2008). However, at higher concentration of Cr is toxic to plants due to much interference with other essential nutrients (Mohanty *et al.*, 2011; and Mohanty and Patra 2016). Chromium is known to affect metabolic processes via, oxidative stress causing chloroplast and pigment alterations (Nakano and Asada 1981; and Suthar *et al.*, 2014).

Horse gram (*Dolichos biflorus* L.) belongs to the subfamily faboideae of family Fabaceae / Leguminosae it is annual herb, partially trailing or climbing, 30-50 cm in height. The crop known as poor men's and a crop of poor resource, is widely grown in India in almost 200-700 mm rainfall situations at a temperature range of 20-35 ° C. It is drought resistant crop and is typically adapted to a wide range of soils. It is grown as sole crop and in number of combinations. The seeds contain crude protein 20.8, pentosan 10.8 and water-soluble gum 2.8%. The presence of antinutritional components such as haemagglutinin and a protease inhibitor. The inhibitor activity decreased during germination. The mean protein value of the seeds is 25.47% which is more or less equivalent to soybean, winged bean and grams. The present investigation has been made to the different level of chromium tolerance and toxicity in Horse gram (*Dolichos biflorus* L).

2. Materials and methods

The seeds of Horse gram *Dolichos biflorus* L. verity (Paiyur-1) seeds were obtained from Local Agro Service Center and the seeds are uniform size and colors were selected for the experimental purpose. The Chromium ($K_2Cr_2O_7$) Potassium dichromate (VI), molecular weight 294.185 g/mol, was obtained from Scientific Equipments Company, Dharmapuri District, Tamil Nadu. The treatment of pot experiments, viz., control (normal soil), 2, 4, 6, 8 and 10 mg/kg^{-1} of Cr incorporated with soil. The horse gram seeds were sown in the each pot to irrigate with normal tap water.

2.1. Seed treatment

The seed treatment was given with carbendazim @ 1 g for 0.5 kg of seed in order to protect from seed born diseases. Pre-sowing irrigation was given to ensure uniform germination. Irrigation was given at two days with due care to avoid excess flooding of water. Uniform irrigation was given for four times a week. Five plant samples were randomly collected at 60DAS and they were used for observations of morphological parameters like root length, shoot length, total leaf area, fresh weight and dry weight of the plants.

2.2. Germination percentage

The number of seeds germinated in each treatment was counted on each and every day up to 7th day after sowing (DAS). The total germination percentage was calculated by using the following formula:

$$Germination\ Percentage = \frac{Total\ number\ of\ seeds\ germinated}{Total\ number\ of\ seeds\ sown} \times 100$$

2.3. Shoot length and root length

Five plants were randomly selected for recorded at 60th DAS of root and shoot length of Horse gram plants. They were measured by using centimeter scale.

2.4. Total leaf area (Kalra and Dhiman, 1977)

Five plant samples were collected at 60th DAS the length and breadth of the leaf samples were measured and recorded. The total leaf area was calculated by using the Kemp's constant.

$$Total\ leaf\ area = L \times B \times K$$

where, *L* - length, *B* - breadth and *K* - Kemp's constant (for dicot - 0.66).

2.5. Biochemical analyses

The photosynthetic pigments such as chlorophyll 'a', 'b' and carotenoid. The biochemical contents starch, amino acid, total sugars and protein, were analyzed on the 60 DAS of experimental plants.

2.6. Chlorophyll (Arnon, 1949)

Five hundred mg of fresh leaf material was ground with a mortar and pestle with 10 mL of 80% acetone. The homogenate was centrifuged at 800 rpm for 15 min. The supernatant was saved and the residue was reextracted with 10 mL of 80% acetone. The supernatant was saved and the absorbance values were read at 645 and 663 nm in a UV-spectrophotometer. The chlorophyll a, chlorophyll b and total chlorophyll contents were estimated and expressed in mg g^{-1} fresh weight basis.

Chlorophyll 'a' = (0.0127) × (O.D 663) – (0.00269) × (O.D 645)
Chlorophyll 'b' = (0.0229) × (O.D 645) – (0.00488) × (O.D 663)
Total chlorophyll = (0.0202) × (O.D 645) + (0.00802) × (O.D 663)

2.7. Carotenoid (Kirk and Allen, 1965)

The same plant extract used for chlorophyll estimation was used for carotenoid estimation. The acetone extract was read at 480 nm in a UV-spectrophotometer. The carotenoid content was calculated by using the following formula and it is also expressed in mg g-1 fresh weight basis.

$$Carotenoid = (O.D\ 480) – (0.114) \times (O.D\ 663) – (0.638) \times (O.D\ 645)$$

2.8. Estimation of protein (Lowry et al., 1951)

One mL of the extract was taken in a 10 mL test tube and 5 mL of reagent 'C' was added. The solution was mixed and kept in darkness for 10 min. Later, 0.5 mL of Folin-phenol reagent was added and the mixture was kept in dark for 30 min. The sample was read at 660 nm in a UV-spectrophotometer.

2.9. Estimation of sugars (Nelson, 1944)

One mL of extract was taken in a 25 mL marked test tube. One mL of reagent 'C' was added. Then, the mixture was heated for 20 min at 100 °C in a boiling water bath, cooled and 1 mL of arsenomolybdate reagents was added. The solution was thoroughly mixed and diluted to 25 mL with distilled water. The sample was read at 520 nm in a UV-spectrophotometer.

2.10. Extraction and estimation of starch (Dubois et al., 1956)

Five hundred mg of plant material was weighed and macerated in a pestle and mortar with 10 mL of 80% ethanol. The sample was centrifuged at 6000 rpm for 15 min. The supernatant was removed and the pellets were extracted with 52% perchloric acid for 30 min at 0 °C. The extract was centrifuged and supernatant was diluted up to 15 times. One mL of diluted sample was mixed with 2 mL of cold anthrone reagent in ice bath and it was boiled for 10 min at 100 °C in a water bath. The content was cooled and the absorbance was read at 630 nm in a UV-spectrophotometer. The starch was calculated by multiplying with 0.9 to the values obtained from standard curve. The starch contents were expressed in mg g^{-1} fresh weight.

2.11. Estimation of amino acids (Moore and Stein, 1948)

One mL of the extract was pipetted out into a test tube. A drop of methyl red indicator was added. The sample was neutralized with 1 mL of 0.1 N sodium hydroxide. To this, 1 mL of ninhydrin reagent was added and mixed thoroughly. The content of the test tube was heated for 20 min in a boiling water bath. Five mL of the diluent solution was added and heated in water bath for 10 min. The tubes were cooled under the running water and the contents were mixed thoroughly. Blank was prepared with 1 mL of distilled water or ethanol. The absorbance was read at 570 nm in a UV-spectrophotometer.

3. Results and discussion

Table 1 shows that the effects of Cr on the seed germination percentage of Horse gram. The maximum seed germination percentage (99%) of Horse gram observed in 2 g / kg^{-1} of Cr treated soil on 7^{th} DAS when compared to other treatments and lowest seed germination (20%) was noted in 10 mg / kg^{-1} of Cr treatment. It has been

Table 1: Phyto toxicity of chromium on the germination and morphological parameters of Horse gram on 60th DAS

Chromium added in the soil (mg kg^{-1})	60th DAS (Day After Sowing)							
	Germi-nation (%) 7th DAS	Root length (cm)	Shoot length (cm)	Leaf area (cm^2 plant^{-1})	Fresh weight (g plant^{-1})	Dry weight (g plant^{-1})	Pigment Content	
							Chl-a	Chl-b
Control	94 ± 4.8	9.4 ± 0.094	17.6 ± 0.88	19.7 ± 0.98	22.5 ± 1.13	6.4 ± 0.32	0.85 ± 0.04	1.36 ± 0.07
2	99 ± 4.95	13.5 ± 0.67	23.3 ±1.165	22.5 ± 1.13	27.2 ± 0.14	9.5 ± 0.475	2.40 ± 0.12	3.55± 0.18
4	85 ± 4.25	8.2 ± 0.41	15.5 ± 0.77	18.7 ± 0.92	16.8 ± 0.84	5.8 ± 0.29	1.23 ± 0.06	2.20 ± 0.11
6	79 ± 3.95	6.5 ± 0.32	10.0 ± 0.05	13.9 ± 0.69	11.8 ± 0.06	3.6 ± 0.18	0.74 ± 0.04	1.45 ± 0.07
8	54 ± 2.7	3.4 ± 0.17	7.9 ± 0.39	10.4 ± 0.05	8.8 ± 0.44	1.2 ± 0.06	0.38 ± 0.02	0.92 ± 0.05
10	20 ± 1.0	2.2 ± 0.11	5.0 ± 0.25	7.0 ± 0.35	5.4 ± 0.27	0.8 ± 0.04	0.22 ± 0.01	0.57 ± 0.02

Note: ± Standard deviation.

difference of opinion that decrease in seed germination may be due to lowering of water potential or through ionic imbalance. HMs may cause degradation of enzymes present inside the seed like δ-amylase and protease which results in decrease in seed germination (Verma and Dubey, 2003; Jun *et al.*, 2009; Heidari and Sarani, 2011; and Gubrelay *et al.*, 2013). Reduced germination percentage of Horse gram at higher Cr level may be attributed to the interference of metal ions which may hinder seed germinations by exerting unfavorable effect in the utilization of major seed reservoirs like starch, α-amylase, an enzyme involved in seed germination to supply sugar, may block the adverse effects (Tantrey and Agnihotri, 2010; and Kranner and Colville, 2011).

Table 1 represent the maximum shoot length (23.3 ± 1.165) root length (13.5 ± 0.67) on 60 DAS respectively was observed at 2 mg / kg^{-1} of Cr treatment. The minimum shoot length (5.0 ± 0.25) root (2.2 ± 0.11) length on observed in 10 mg / kg^{-1} of Cr treatment. Cr was found to be more toxic affecting shoot and root length .The reduction in the shoot height might be mainly due to the reduced root growth and consequent lesser nutrient and water transport to the above parts of the plants. In addition to this, Cr transport to the aerial part of the plant can have a direct impact on cellular metabolism of shoots contributing to the reduction of plant height (Shanker *et al.*, 2005; and Joshi *et al.*, 2019).

Root was found to be more affected than shoot. This is due to the fact that HMs (Cr) accumulated on root due to binding of metals (Cr) on the cell wall of root and retard cell division and cell elongation and accumulation of metals with in roots reduces the mitotic rate in meristematic zone particularly by blocking metaphase in meristematic cells and thereby reduces their length (Shanker and Pathmanabhan, 2004; and Sundaramoorthy *et al.*, 2010). General decreased root growth due to Cr toxicity could be due to inhibition of root cell division / root elongation or to the extension of cell cycle in the roots. The reason of the high accumulation in roots of the plants could be because Cr is immobilized in the vacuoles of the root cells, thus rendering it less toxic, which may be a natural toxicity response of plant, and also HMs have been reported to impair the growth of new roots and seedling establishment (Lin and Xing, 2007; and Singh *et al.*, 2013b).

Decrease in leaf area of horse gram at higher concentration of Cr may be due to decreased activities of many enzymes involved in the fixation of CO_2, changes in the thylakoid organization, reduction of chlorophyll contents and inhibition of photosynthetic activities and disturbing the interaction of chlorophyll molecules into the stable complex (Fodar *et al.*, 1996; and Faizan *et al.*, 2011). The fresh and dry weight of Horse gram plants elevated in various levels of Cr is furnished in (Table 1). The Cr at 2 mg kg^{-1} level in the soil increased the fresh and dry weight of experimental plants and decreased at high levels of Cr (4-10 mg kg^{-1}) on 60 DAS. The identical reports was found that dry matter production was severely affected by Cr (VI) concentrations above 2.5 Ag mL^{-1} in nutrient medium (Li *et al.*, 2005). Zurayk *et al.* (2001) reported that salinity and Cr (VI) interaction

caused a significant decrease in the dry biomass accumulation of *Portulaca oleracea*. Cauliflower (cv. Maghi) when cultivated at 0.5 mM Cr (VI) restricted dry biomass (Chatterjee and Chatterjee, 2000).

They proposed that reduction in biomass production under the influence of Cr may be due to the impairment of uptake and translocation of nutrients and water in aerial parts of plants. It is consider that after absorption root passes Cr to shoot through translocation. Thus, roots are less affected than shoots under heavy metal stress. During seedling growth hydrolysis of food reserves takes place which is carried out by hydrolytic enzymes. So the activities of hydrolytic enzymes might be affected and the food did not reach to the radical and plumule leading to the reduction in seedling growth (Farooqi *et al.,* 2009; Sundaramoorthy, 2009; Singh *et al.,* 2013a; and Kundu *et al.,* 2018). Under lower application of Cr improved root system helped the plants in better absorption of water and other nutrients dissolved in the soil and consequently improved the growth of different organs and the entire plant of horse gram.

Effect of Cr on the pigment and biochemical content of Horse gram was represented in Table 2. Pigment content such as chlorophyll 'a', chlorophyll 'b', carotenoid, and biochemical content like, viz., starch, amino acid, sugars and protein content of Horse gram leaves increased at lower concentration (2 mg kg^{-1}) and decreased further with an increase in the Cr level (4-10 mg kg^{-1}). The chlorophyll 'a' and 'b' content increased at 2 mg kg^{-1} of the Cr level (2.40 ± 0.12; 3.55± 0.18) and decreased (0.22 ± 0.01; 0.57 ± 0.02) chlorophyll content respectively. The chlorophyll content decreased with increasing concentration of Cr form the experimental results of the present investigation the increased chlorophyll content at the lower level of Cr was obviously due to better growth of horse gram.

Table 2: Phyto toxicity of chromium on biochemical content of Horse gram. on 60th DAS

Chromium added in the soil (mg kg^{-1})	60th DAS (Day After Sowing)				
	Carotinoid	Total sugars	Starch	Amino acids	Protein
Control	1.20 ± 0.06	2.60 ± 0.13	3.18 ± 0.159	3.75 ± 0.1875	3.12 ± 0.156
2	2.60 ± 0.13	3.86 ± 0.19	5.12 ± 0.256	5.93 ± 0.2965	6.28 ± 0.314
4	1.04 ± 0.052	2.00 ± 0.04	3.40 ± 0.17	4.62 ± 0.231	5.12 ± 0.256
6	0.82 ± 0.041	1.65 ± 0.08	1.85 ± 0.0925	3.32 ± 0.166	3.68 ± 0.234
8	0.63 ± .0315	0.72 ± 0.37	0.88 ± 0.044.	2.02 ± 0.101	1.28 ± 0.164
10	0.40 ± 0.02	0.28 ± .014	0.51± 0.0255	1.24 ± 0.062	0.42 ± 0.105

Note: ± Standard deviation.

The decrease in chlorophyll 'a' 'b' content due to Cr could be due to the destabilization and degradation of the proteins of the peripheral part. The inactivation of enzymes involved in the chlorophyll biosynthetic pathway could also contribute to the general reduction in chlorophyll content in plants under Cr stress. Moreover, Cr can interfere by substituting Mg at the active site of the enzyme (Vajpayee *et al.,* 2000; Hadif *et al.,* 2015; and Ma *et al.,* 2016). The more pronounced effect of Cr (VI) on PS I than on PS II activity in isolated chloroplasts has been reported by (Bishnoi *et al.,* 1993; Ghani 2011; Reale *et al.,* 2016; Ding *et al.,* 2016; and Li *et al.,* 2018).

Li *et al.* (2018) as suggested by decrease in protein content at higher Cr application may be due to the enhanced rate of protein denaturation. Inhibitory effect of metals on protein synthesis may be due to the increased protein hydrolysis by catalytic activity and severe oxidative stress imposed under metals stress conditions (Kumar *et al.,* 2011; and Anjum *et al.,* 2017). The increased chlorophyll content at lower level of Cr was obviously due to better growth, they can directly destroy the structure and function of chloroplast by binding with $^{-}$SH group of enzyme and over all chlorophyll biosynthesis through Mg^{2p}, Fe^{2p} or Zn^{2p} (Singh, 2001). The decrease in biomass in excess of Cr might be due to low protein formation, resulting in the inhibition of photosynthesis, as well as hampering carbohydrate translocation (Manivasagaperumal *et al*., 2011; and Ali *et al.,* 2015).

The lesser sugar content in the plant at high concentrations of Cr treatments implies the deranged metabolism and poor translocation of sugars and starch metabolites to the growing parts. The decline in sugar formation may be associated with reduced rates of photochemical activities and chlorophyll formation. Loss of sugar construction may also be due to the change of sugar into energy when the plants were metal stressed (Outridge and Noller, 1991; Sundarmoorthy *et al.,* 2015; and Ibrahim *et al.,* 2017).

4. Conclusion

From the above experiments, it can be concluded that the soil polluted with Cr toxicity on seed germination, morphology, chlorophylls and biochemical content of Horse gram. Our results suggest that the 2 mg kg^{-1} level of Cr in the soil was beneficial for the growth of Horse gram plants. The level of Cr in the soil above 4 mg kg^{-1} proved to be toxic to Horse gram. The results indicated that the 2 mg kg^{-1} Cr level for increasing the morphology and biochemical content of Horse gram plants.

Conflicts of Interest

There is no conflict of interests between the authors of this manuscript.

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