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Research Article

Chromium phytoaccumulation and tolerance potential of Coffe Pod: *Cassia tora* (L.) Roxb. grown in chelate and fertilizer assisted soil

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Abstract: Chromium is a heavy element which plays a vital role in metabolism of cholesterol fat and glucose while, at higher concentration it is toxic and carcinogenic. Pot culture experiments using hexavalent Chromium (Cr^{+6}) with combination of chelater and fertilizer were performed to assess the growth and Chromium bioaccumulation in 60 days old plants of Coffe pod. Root and shoot length, fresh and dry matters considerably increased in fertilizer application than other treatments. Chromium bioaccumulation was more in roots than leaves and stems. Bio-Concentration Factor (BCF), Total Accumulation Rate (TAR) indicated highest values i.e., 0.203 and 2.514 respectively using Cr^{+6}-EDTA-FRZ (50ppm) whereas Transportation Index (TI) was highest (2.510) using 50ppm of Cr+6.

Key words: Coffe pod; chelater, Cr-phytoaccumulation, Total Accumulation Rate

Introduction

Pollution due to heavy metal is a matter of growing concern because of their toxicity to all forms of life. Heavy metals mostly accumulate in soil and water and these are threat to plants since they are rooted to soil and hence get maximum exposure. Heavy metals are a threat to plants and animals because they are non-biodegradable and their accumulation readily reaches toxic levels (Khan *et al.,* 2009). Chromium is an important toxic environmental pollutant. Hexavalent chromium ($Cr^{6+)}$ stress is one of the major abiotic stress problems in chromite mining sites. Plants that are exposed to environmental contamination by chromium are affected in diverse ways, including a tendency to suffer metabolic stress. The studies on attenuation of toxic effects of chromium using plants in Cr^{+6} amended in soil are limited (Chaturvedi *et al.,* 2015).

Phytodetoxification is a process which brings detoxification of heavy metals through plant-based chelation, reduction, and oxidation mechanisms. (Salt *et al.,* 1998; Zayed and Terry, 2003; Panda and Patra, 1997; Mohanty and Patra, 2011; Chaturvedi *et al.,* 2015). Coffe pod (*Cassia tora*) is a wild legume in tropics and few reports suggest its role as hyper accumulator activity (Siringoringo, 2000; Shirbhate and Malode, 2012). In this context, pot culture study with combined effect of chromium, chelater and fertilizer amended soils was designed to analyze the phytotoxic effects of Cr^{+6} on bioavailability in *Cassia tora* (Coffe pod). Further, efforts were made to verify the effects of fertilizer, chelater with combination of chromium on growth and tolerance behavior in response to Cr^{+6}.

Materials and Methods

Plant Material and Growth.

Cassia tora (L.) Roxb. (Coffe pod) seeds were collected from the mature pods of the Cassia tora plants grown as a wild weed from Utkal University campus. The seeds were surface sterilized with 0.1% mercuric chloride (w/v) for 5 minutes followed by thoroughly washing in tap water and distilled water before use for sowing in culture pots for plant growth and analysis.

The seedlings were grown under in each poly-pots containing 3kg dry garden soil (sandy loam) and green manure in the ratio of 3:1.Ten days old seedlings were further amended in different manners i.e., with Cr^{+6} (50ppm), Fertilizer, $Cr^{+6(}$50ppm)+Fertilizer, Cr^{+6}-EDTA (50ppm) and Cr^{+6}-EDTA(50ppm)+Fertilizer. The application of fertilizer in the respective pots was one gram/3 kg of dry soil. The commercial fertilizer used in the experiments indicates the combinations as follows NPK Fertilizer.

Growth Culture Experiment

The plant growth analyses was undertaken by measuring root and shoot length, fresh weight and dry weight of roots and shoots of 60 days plants grown in Chromium amended soils using chelater and ferlizer.

Analyses of Cr Bio-availability in Plant Tissues:

Total Cr content in root, stem and leaves of 60 days plants of *Cassia tora, was* determined according to Bonet *et al.* (1991). Before analysis of total Cr content, the roots were rinsed with 0.01N HCl followed by washing with distilled water for

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removing mixed Fe and Cr hydrous oxides, which may have precipitated on the root surfaces. Root, stem and leaves of 60 days treated *Casia tora* plants from different pots of were oven dried and grinded separately to fine powders. Nitric acid and Perchloric acid in the ratio of 10:1 as acid mixture was used for digestion of powdered plant samples for extraction of total Cr content of roots, stems and leaves using MDS-8-Microwave Digestion Unit. The acid digested solutions were filtered and the final volume was made up to 100 ml. Total Cr bioaccumulation was estimated from different plant parts using extracted liquid samples in an Atomic Absorption Spectrophotometer (Perkin Elmer, AAanalyst 200, USA).

Plant mass were analyzed for BCF (Bio Concentration Factor), Total AccumulationRate (TAR) and Transportation index (Ti) as per following method (Zurayk *et al.,* 2002; Ghosh and Singh, 2005).

BCF=Average Cr concentration in plant tissue (mg/kg)
Initial concentration of chromium in the soil (mg/kg)
TAR= (Shoot concentration × Shoot biomass + Root concentration × Root biomass)
[(Shoot biomass + Root biomass) × days of growth]

N.B.: TAR (mg/kg/day), Biomass (gm dry wt.) and Concentration (mg/kg dry matter)
Ti = Cr concentration of leaves (mg/kg) X 100
Cr content of root (mg/kg)

Statistical analysis
Soil and plants will be sampled and statistically analyzed. The data will be subjected to analysis of variance (ANOVA) at $P < 0.05$ and $P < 0.01$ and standard deviation values using triplicate data and the data presented in the figures and tables are mean ± SEM (Standard Error of Mean) of three replicates.

Results and Discussion

The plants grown with supplemented fertilizer only showed increased root length, shoot length, fresh weight of root and shoot than Cr^{+}, Cr^{+6}-EDTA and Cr^{+6}-EDTA-FRZ. The increased biomass could be attributed to enhanced nutrients supply by fertilizers in absence of Cr^{+6}. However, the height and fresh weight were reduced at higher concentration of Cr^{+6} (50 ppm). This is probably due to the reason that toxicity of heavy metals significantly inhibited root vitality, checking plant roots from absorbing mineral ions and leading to reduction of plant growth and development (Datta *et al.,* 2011). The growth responses of the plants have been shown in Plate No.1, Figure 1, Figure 2 and Figure 3 respectively.

Plate 1. Photograph showing comparative growth of 60 days old *Cassia tora* plants using different types of Cr applications.

Left to right: Treatments: Control, Fertilizer, Cr-50ppm, Cr-Fertilizer(50ppm), Cr-EDTA (50 ppm), Cr-EDTA-Fertilizer (50 ppm)

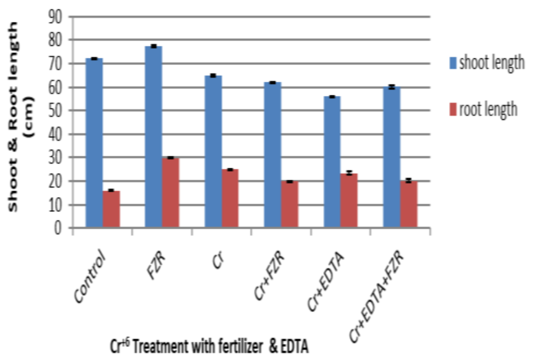

Figure 1. Effect of Cr^{+6} treatment with fertilizer & EDTA on shoot & root length of 60 days *Cassia tora* plants. Abbrv.Used: FZR-Fertilizer, Cr-Chromium Treatments: Control, Fertilizer, Cr-50ppm, Cr-Fertilizer(50ppm), Cr-EDTA (50 ppm), Cr-EDTA-Fertilizer(50 ppm).

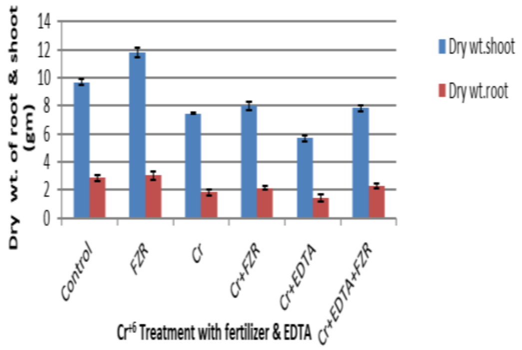

Figure 3. Effect of Cr^{+6} treatment with fertilizer & EDTA on Dry weight of shoot & root of 60 days *Cassia tora* plants.Abbrv. Used: FZR-Fertilizer, Cr-Chromium Treatments: Control, Fertilizer, Cr-50ppm, Cr-Fertilizer (50ppm), Cr-EDTA (50 ppm), Cr-EDTA-Fertilizer(50 ppm)

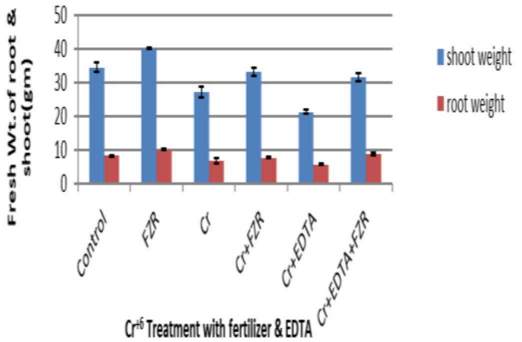

Figure 2. Effect of Cr^{+6} treatment with fertilizer & EDTA on fresh wt. of shoot & root of 60 days *Cassia tora* plants.Abbrv.used: FZR-Fertilizer, Cr-ChromiumTreatments: Control, Fertilizer, Cr-50ppm, Cr- Fertilizer (50ppm), Cr-EDTA (50 ppm), Cr-EDTA-Fertilizer (50 ppm)

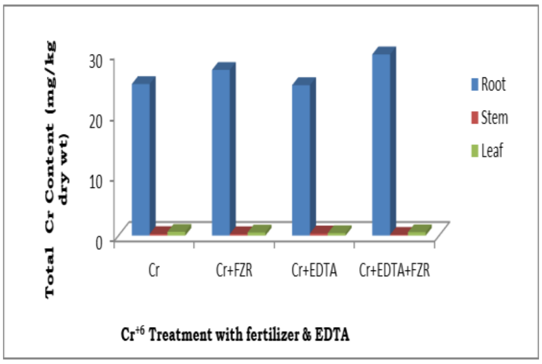

Figure 4. Effect of Cr^{+6} Treatment with fertilizer & EDTA on total Cr bioaccumulation (mg/kg dry mass) of 60 days *Cassia tora* plants.Abbrv.Used: FZR-Fertilizer,Cr-Chromium
Treatments: Control, Fertilizer, Cr-50ppm, Cr-Fertilizer (50ppm), Cr-EDTA (50 ppm), Cr-EDTA-Fertilizer (50 ppm)

Table 1. Transportation index (Ti), Bio Concentration Factor (BCF), and Total Accumulation Rate (TAR) of 60 days old *Cassia tora* plant.

Treatment (ppm)	Transportation Index (Ti)	Bio-Concentration Factor (BCF)	Total Accumulation Rate (TAR) ($mg\ kg^{-1}day^{-1}$)
Control	0.00	0.00	0.00
Cr^{+6}(50 ppm)	2.510	0.172	2.021
Cr^{+6}-FRZ(50 ppm)	1.915	0.186	2.362
Cr^{+6}-EDTA(50 ppm)	1.677	0.170	1.574
Cr^{+6}-EDTA-FRZ (50ppm)	1.851	0.203	2.514

Chromium content was higher in root than that of shoot (Fig. 4). Cr accumulated more in roots as compared to aerial parts during plant growth and development. The maximum Cr accumulation was observed in root (29.806) using Cr^{+6}-EDTA-FRZ(50ppm). Cr accumulation in stems was less (0.136) as compared to root and leaves. The aerial parts of the coffe pod plants showing less Cr as compared to roots is a familiar feature as noted by other workers in different plant species (Ghosh and Singh 2005; Erenoglu *et al.,* 2007; Mohanty and Patra 2011). High Cr accumulation in roots and less uptake to aerial portion of the plant is the most common metal tolerance character evidenced by (Ghosh and Singh, 2005).The metal uptake in plants is fixed to a chemiosmotic route diagonal to the membrane of intact root cells (Zayed and Terry, 2003). Bio-Concentration Factor (BCF), Total Accumulation Rate (TAR) indicated highest values i.e. 0.203 and 2.514 respectively using Cr^{+6}-EDTA-FRZ (50ppm) whereas Transportation Index (TI) was highest (2.510) using 50ppm of Cr^{+6} (Table 1).

The present study confirms that Coffe pod(*Cassia tora)* is capable of up accumulating Cr in a moderate way and can be treated as a hyperaccumulater plant. The application of chelaters and fertilizers restores the damage caused by heavy metal Cr and shows increased plant growth and development and accumulation of metals.

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