EFFECT OF CHROMIUM (CR) INCOW PEA (*Vigna unguiculata*) (L.) WALP.

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ABSTRACT

Chromium is found in all phases of the environment, including air, water and soil. The contamination of environment by chromium has become a major area of concern. Chromium effluent is highly toxic to plant and is harmful to their growth and development. In present study, a pot experiment was carried out to assess the germination study of different varieties of COW PEA (*Vigna unguiculata*) (L.)Co-1,Co-2,Co-3,Co-4,Co-7,Vamban-1,Vamban-2at different concentration of chromium(Control-normal tab water,and different concentration of chromium such as 2.5mg,5mg,7.5mg,10mg,12.5mg. The phytotoxic effect of chromium was observed on seed germination, shoot length,root length,no of leaf,fresh weight,dry weight, chlorophyll-a, chlorophyll-b content and tolerance indices of Cow Pea. All results when compared with control show that chromium metal adversely affects the growth of Cow Pea by reducing seed germination and decreasing seedling growth. The toxic effects of chromium metal to seed germination and young seedling are arranged in order of inhibition as: 0.5, 2.5, 5,7.5, 10.12.5 $mg \cdot kg^{-1}$ respectively. The toxicity of chromium metal to young seedling and their effects on chlorophyll content were increased with higher concentration of chromium in the soil system. The major inhibitory effect of chromium in Cow Pea seedling was determined as stress tolerance index (%). The present study represents that the seed and seedling of Cow Pea has potential to counteract the deleterious effects of chromium metal in soil.

Keywords: Chromium; Phytotoxicity; Germination; Growth; Biochemical Attributes

1. Introduction

Naturally occurring chromium is usually present as soil ,Chromium (Cr) is one of the heavy metals that cause severe environmental contamination in soil, sediments and groundwater. Wastes coming from chromium related industries, such as tanneries, electroplating and mining activities contribute to most of the chromium contamination;

Several methods are already being used to clean up the environment from these kinds of contaminants, but most of them are costly and far away from their optimum performance. Mycophytoremedial is a term functional to a group of technologies that use plants to reduce, remove, degrade, or immobilize environmental toxins, primarily those of anthropogenic origin, with the aspire of restoring area sites to a condition useable for private or public applications. Arbuscular mycorrhizal fungi (AMF) are amongst the most common soil fungi and the majority of plant species have associations with AM fungal species (Selvaraj & Chellappan 2006). The AM fungi are significant in the remediation of contaminated soil as accumulation (Jamal *et al.*, 2002). The external mycelium of AM fungi allows for wider exploration of soil volumes by spreading beyond the root exploration zone (Khan *et al.*, 2000), thus providing access to greater quantities of heavy metals present in the rhizosphere. Higher concentrations of metals are also stored in mycorrhizal structures in the root and in fungal spores. AMF can also increase plant establishment and growth despite high levels of soil heavy metals due to improved nutrition (Taylor and Harrier, 2001), water availability (Auge, 2001), and soil aggregation properties (Kabir and Koide, 2000) associated with this symbiosis.

2. MATERIALS AND METHODS

2.1. Plastic cup Experiments

The seeds Cow pea (Co-1,Co-2,Co-3,Co-4,Co-7,Vamban-1,Vamban-2) was obtained from Tamilnadu Agricultural University (TNAU), Coimbatore and Tamilnadu. The uniform seeds are selected for the experimental purpose. Source of Cr (Potassium dichromate ($K2Cr2O7$) stock solution prepared by dissolving the molecular weight of (Cr) and different concentrations *viz.*, (Control, 0.5, 2.5, 5, 7.5, 10.12.5 mg/kg−1) of (Cr) the solution were prepared freshly at the time of experiments. The plastic cups were filed with 1 Kg of garden soil, selected cow pea seeds were sown in the plastic cup and one set of plastic cup irrigated with normal tap water was maintained as the control.

2.2. Shoot length and root length (cm/seedling)

Five plants from each plastic cup were randomly selected for 14th days of seedlings recorded the shoot length and root length of experimental plants. They were measured by using centimeter scale (Cm).

2.3. Total leaf area

Five plant samples were collected at 14th day sampling seedlings and the length and breadth of the leaf samples were measured and recorded. The total leaf area was calculated by using the Kemps constant [10].

Total leaf area = L × B × K

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

2.4. Fresh weight and dry weight (g/seedling)

Five plant samples were randomly selected at 14th day seedlings. Their fresh weight was taken by using an electrical single pan balance. The fresh plant materials were kept in a hot air over at 80°C for 24 hr and then their dry weight were also determined.

2.5. Vigour index

Vigour index of the seedlings were calculated by using the formula proposed by [11].

Vigour index = Germination percentage × seedling length.

2.6. Tolerance index

Tolerance index of the seedlings were calculated by using the formula proposed by [12].

control in root longest of length Mean treatment in root longest of length Mean index Tolerance

2.7. Phytotoxicity

The percentage of phytotoxicity of effluent was calculated by using the formula proposed by

Percentage of phytotoxicity =Shoot or root length of control

Shoot or root length of treatment X100 Shoot or root length of control

100 control of length Radicle test of lenght Radicle - control of length Radicle .

2.8. Chlorophyll (Arnon, 1949)

Five hundred mg of fresh leaf material was ground in a mortar and pestle with 10 mL of 80 per cent acetone. The homogenate was centrifuged at 800 rpm for 15 min. The supernatant was saved. The residue was re-extracted with 10 mL of 80 per cent acetone. The supernatant was saved and the absorbance values were read at 645 and 663 nm using a UV-Spectrophotometer (Hitachi). 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)

3. RESULT

The seed germination percentage of Cowpea varieties were recorded on 7^{th} day after seed sowing which are given in Table-1. The maximum seed germination percentage of Cowpea varieties was found in Co-7 as compare to others varieties of Cowpea in all treatment concentrations . The lowest seed germination was found in Co-2 variety.

An influence of chromium on the shoot length of Cowpea varieties were presented in Table-2.Among Cowpea varieties the highest shoot length was recorded in Co-7 with comparison of other varieties. The lowest shoot length was recorded in Co-2 variety.

The effect of chromium on the root length Cowpea varieties were shown in Table-3. The maximum root length was observed in Co-7 compare to other varieties. The minimum root length was observed in Co-2 variety.The effect of chromium on the no of leaves, fresh weight and dry weight of Cowpea varieties is shown in Table-4,5 and 6. The variety Co-7 was shown in better result in all analyzed parameters compare to other varieties.

The influence of chromium on different varieties of Cow pea of Vigour index, Tolerance index and Phytotoxicity are depicted in Table 7,8 and 9. The maximum Vigour index, Tolerance index and Phytotoxicity were observed in Co-7 as compare to other varieties. The photosynthetic pigment such as chlorophyll a, b and total chlorophyll content of different varieties Cow pea are given in Table- 10,11 and 12. The highest photosynthetic pigment content was found in Co-7 as compare to other varieties.

Chromium Treatments	**Seed Germination Percentage (DAS)**						
	CO-1	**CO-2**	**CO-3**	**CO-4**	**CO-7**	**Vamban-1**	**Vamban-2**
Control	92±2.76	91±2.73	92±2.76	95 ±2.85	**98 ±2.94**	95 ±2.85	93±2.79
2.5 mg/kg	88±2.64	80±2.4	85±2.55	84±2.52	**90 ±2.7**	83±2.49	84±2.52
5 mg/kg	75±2.25	69±2.07	70±2.1	80 ±2.4	**82±2.46**	78 ±2.34	76 ±2.28
7.5 mg/kg	66±1.98	52±1.56	58±1.74	74 ±2.22	**72 ±2.16**	71 ±2.13	70 ±2.1
10 mg/kg	32±0.96	31±0.93	30±0.9	61 ±1.83	**61±1.83**	62 ±1.86	60±1.8
12.5 mg/kg	29±0.87	24±0.72	25±0.75	34 ±1.02	**42 ±1.26**	35±1.05	34±1.02

Table.1 Effect of Chromiumon seed germination percentage of *Vigna unguiculata* (L.) WALP. On 7th DAS

Chromium Treatments	**Shoot length**						
	CO-1	**CO-2**	**CO-3**	**CO-4**	**CO-7**	**Vamban-1**	**Vamban-2**
Control	12.2±0.366	11.6±0.348	12.0±0.36	12.8±0.384	**14.2 ±0.426**	13.1±0.393	13.4±0.402
2.5 mg/kg	11.3±0.339	9.8±0.294	10.5±0.315	11.2±0.336	**13.8 ±0.414**	12.6±0.378	12.1±0.363

5 mg/kg	9.5±0.285	7.4±0.222	8.0±0.24	9.4±0.282	**10.2 ±0.306**	9.6±0.288	10.0±0.3
7.5 mg/kg	7.2±0.216	6.1±0.183	6.3±0.189	6.8±0.204	**9.0 ±0.27**	7.2±0.216	8.4±0.252
10 mg/kg	4.6±0.138	3.6±0.108	3.2±0.096	4.5±0.135	**7.5 ±0.225**	5.3±0.159	5.2±0.156
12.5 mg/kg	2.8±0.084	2.4±0.072	2.6±0.078	3.2±0.096	**5.2 ±0.156**	3.6±0.108	3.6±0.108

Table.2 Effect of Chromiumon shoot length of *Vigna unguiculata* (L.) WALP. On 7th DAS

Chromium Treatments	**Root length**						
	CO-1	**CO-2**	**CO-3**	**CO-4**	**CO-7**	**Vamban-1**	**Vamban-2**
Control	4.3±0.129	3.8±0.114	4.5±0.135	4.7±0.141	**6.1±0.183**	4.3±0.129	4.3±0.129
2.5 mg/kg	3.2±0.096	3.2±0.096	3.4±0.102	3.6±0.108	**5.2 ±0.156**	3.7±0.111	3.4±0.102
5 mg/kg	3.0±0.09	2.4±0.072	3.1±0.093	3.0±0.09	**4.0 ±0.12**	3.0±0.09	2.8±0.084
7.5 mg/kg	3.0±0.09	2.0±0.06	2.5±0.075	2.7±0.081	**4.0 ±0.12**	2.6±0.078	1.6±0.048
10 mg/kg	2.1±0.063	1.6±0.048	1.8±0.054	2.3±0.069	**3.4±0.102**	2.0±0.06	0.8±0.024
12.5 mg/kg	2.1±0.063	1.2±0.036	1.5±0.045	2.0±0.06	**2.8±0.084**	1.7±0.051	0.5±0.015

Table.3 Effect of Chromiumon Root length of *Vigna unguiculata* (L.) WALP. On 7th DAS

Chromium Treatments	**No. of Leaves**						
	CO-1	**CO-2**	**CO-3**	**CO-4**	**CO-7**	**Vamban-1**	**Vamban-2**
Control	2±0.06	2±0.06	2±0.06	2±0.06	**2±0.06**	2±0.06	2±0.06
2.5 mg/kg	2±0.06	2±0.06	2±0.06	2±0.06	**2±0.06**	2±0.06	2±0.06
5 mg/kg	2±0.06	2±0.06	2±0.06	2±0.06	**2±0.06**	2±0.06	2±0.06
7.5 mg/kg	2±0.06	2±0.06	2±0.06	2±0.06	**2±0.06**	2±0.06	2±0.06

10 mg/kg	1±0.03	1±0.03	1±0.03	1±0.03	**2±0.06**	1±0.03	1±0.03
12.5 mg/kg	-	-	-	-	**1±0.03**	-	-

Table.4. Effect of Chromiumon No. of leaves of *Vigna unguiculata* (L.) WALP. On 7th DAS

Chromium Treatments	**Fresh weight (g.fr.wt)**						
	CO-1	**CO-2**	**CO-3**	**CO-4**	**CO-7**	**Vamban-1**	**Vamban-2**
Control	8.4±0.252	7.9±0.237	8.0±0.24	8.5±0.255	**10.7 ±0.321**	8.6±0.258	8.6±0.258
2.5 mg/kg	5.7±0.171	5.1±0.153	5.2±0.156	5.7±0.171	**8.1±0.243**	6.8±0.204	7.2±0.216
5 mg/kg	4.2±0.126	4.0±0.12	4.2±0.126	4.8±0.144	**7.2 ±0.216**	5.1±0.153	6.4±0.192
7.5 mg/kg	3.0±0.09	2.5±0.075	3.0±0.09	4.1±0.123	**5.4 ±0.162**	4.2±0.126	5.2±0.156
10 mg/kg	2.4±0.072	1.7±0.051	2.1±0.063	3.4±0.102	**3.0 ±0.09**	3.0±0.09	2.8±0.084
12.5 mg/kg	0.8±0.024	0.6±0.018	1.3±0.039	2.0±0.06	**2.8 ±0.084**	1.5±0.045	1.9±0.057

Table.5. Effect of Chromiumon fresh weight of *Vigna unguiculata* (L.) WALP. On 7th DAS

Chromi Treatme	**Dry weight (g.dr.wt)**						
	CO-1	**CO-2**	**CO-3**	**CO-4**	**CO-7**	**Vamban-1**	**Vamban-2**
Control	3.13±0.0939	3.00±0.09	3.08±0.0924	3.15±0.094	**4.90±0.147**	3.19±0.095	3.24±0.097
2.5 mg/kg	2.40±0.072	2.18±0.0654	2.23±0.0669	2.58±0.077	**3.77±0.113**	2.68±0.080	2.74±0.082
5 mg/kg	1.02±0.0306	0.94±0.0282	0.99±0.029	1.05±0.031	**2.28±0.068**	1.40±0.042	1.51±0.045
7.5 mg/kg	0.80±0.024	0.74±0.0222	0.76±0.0228	0.92±0.027	**1.23±0.036**	0.97±0.029	1.00±0.03
10 mg/kg	0.52±0.0156	0.41±0.0123	0.52±0.015	0.59±0.017	**0.69±0.020**	0.61±0.018	0.69±0.020

12.5 mg/kg	0.20±0.006	0.15±0.0045	0.24±0.0072	0.28±0.008	**0.31±0.009**	0.22±0.006	0.28±0.008

Table.6. Effect of Chromiumon dry weight of *Vigna unguiculata* (L.) WALP. On 7th DAS

Chromi Treatme	**chlorophyll 'a' (mg.fr.wt)**						
	CO-1	**CO-2**	**CO-3**	**CO-4**	**CO-7**	**Vamban-1**	**Vamban-2**
Control	1.045±0.031	1.002±0.030	1.093±0.032	1.170±0.035	**1.635±0.049**	1.285±0.038	1.420±0.042
2.5 mg/kg	0.735±0.022	0.713±0.021	0.789±0.023	0.802±0.024	**0.978±0.029**	0.880±0.026	0.904±0.027
5 mg/kg	0.417±0.012	0.395±0.011	0.432±0.012	0.600±0.018	**0.702±0.021**	0.653±0.019	0.697±0.020
7.5 mg/kg	0.187±0.005	0.172±0.005	0.192±0.005	0.213±0.006	**0.536±0.016**	0.302±0.009	0.386±0.011
10 mg/kg	0.089±0.002	0.071±0.002	0.095±0.002	0.108±0.003	**0.384±0.011**	0.170±0.005	0.193±0.005
12.5 mg/kg	0.027±0.00081	0.021±0.0006	0.035±0.001	0.046±0.001	**0.179±0.005**	0.095±0.002	0.106±0.003

Table.7. Effect of Chromiumon chlorophyll 'a' of *Vigna unguiculata* (L.) WALP. On 7th DAS

Chromium Treatments	**chlorophyll 'b' (mg.fr.wt)**						
	CO-1	**CO-2**	**CO-3**	**CO-4**	**CO-7**	**Vamban-1**	**Vamban-2**
Control	0.670±0.020	0.622±0.018	0.692±0.020	0.708±0.021	**0.992±0.029**	0.722±0.021	0.763±0.022
2.5 mg/kg	0.511±0.015	0.470±0.014	0.574±0.017	0.612±0.018	**0.870±0.026**	0.645±0.019	0.690±0.020
5 mg/kg	0.302±0.009	0.287±0.008	0.330±0.009	0.461±0.013	**0.725±0.021**	0.520±0.015	0.573±0.017
7.5 mg/kg	0.220±0.006	0.201±0.006	0.254±0.007	0.305±0.009	**0.462±0.013**	0.409±0.012	0.312±0.009
10 mg/kg	0.138±0.004	0.119±0.003	0.165±0.004	0.189±0.005	**0.201±0.006**	0.186±0.005	0.155±0.004
12.5 mg/kg	0.075±0.002	0.066±0.001	0.083±0.002	0.102±0.003	**0.160±0.004**	0.115±0.003	0.128±0.003

Table.8. Effect of Chromiumon chlorophyll 'b' of *Vigna unguiculata* (L.) WALP. On 7th DAS

Chromium Treatments	Total chlorophyll (mg.fr.wt)						
	CO-1	**CO-2**	**CO-3**	**CO-4**	**CO-7**	**Vamban-1**	**Vamban-2**
Control	1.112±0.033	1.624±0.048	1.785±0.053	1.878±0.056	**2.627±0.078**	2.007±0.060	2.112±0.063
2.5 mg/kg	1.306±0.039	1.183±0.035	0.863±0.025	1.414±0.042	**1.848±0.055**	1.525±0.045	1.594±0.047
5 mg/kg	0.719±0.021	0.682±0.020	0.762±0.022	1.061±0.031	**1.427±0.042**	1.173±0.035	1.27±0.038
7.5 mg/kg	0.407±0.012	0.421±0.012	0.446±0.013	0.518±0.015	**0.998±0.029**	0.711±0.021	0.698±0.020
10 mg/kg	1.027±0.030	0.29±0.008	0.26±0.007	0.297±0.008	**0.585±0.017**	0.356±0.010	0.348±0.010
12.5 mg/kg	0.345±0.010	0.087±0.002	0.118±0.003	0.148±0.004	**0.336±0.010**	0.21±0.006	0.234±0.007

Table.9. Effect of Chromiumon Total chlorophyll of *Vigna unguiculata* (L.) WALP. On 7th DAS

Chromium Treatments	Vigour index						
	CO-1	**CO-2**	**CO-3**	**CO-4**	**CO-7**	**Vamban-1**	**Vamban-2**
Control	1807±54.21	1760±52.8	1976±59.28	1990±59.7	**2112±63.36**	1982±59.46	1934±58.02
2.5 mg/kg	1629±48.87	1602±48.06	1648±49.44	1692±50.76	**1724±51.72**	1670±50.1	1610±48.3
5 mg/kg	1052±31.56	983±29.49	992±29.76	1002±30.06	**1310±39.3**	1105±33.15	1096±32.88
7.5 mg/kg	845±25.35	809±24.27	823±24.69	856±25.68	**920±27.6**	810±24.3	729±21.87
10 mg/kg	370±11.1	320±9.6	349±10.47	390±11.7	**482±14.46**	402±12.06	486±14.58
12.5 mg/kg	188±5.64	179±5.37	195±5.85	199±5.97	**208±6.24**	188±5.64	174±5.22

Table.10. Effect of Chromiumon vigour index of *Vigna unguiculata* (L.) WALP. On 7th DAS

Chromium Treatments	Tolerance index						
	CO-1	CO-2	CO-3	CO-4	CO-7	Vamban-1	Vamban-2
Control	-	-	-	-	-	-	-
2.5 mg/kg	1.326±0.039	1.182±0.035	1.389±0.041	1.228±0.036	**1.647±0.049**	1.132±0.033	1.119±0.033
5 mg/kg	0.640±0.019	0.603±0.018	0.692±0.020	0.708±0.021	**0.952±0.028**	0.665±0.019	0.621±0.018
7.5 mg/kg	0.328±0.009	0.311±0.009	0.330±0.009	0.374±0.011	**0.622±0.018**	0.354±0.010	0.345±0.010
10 mg/kg	0.214±0.006	0.202±0.006	0.234±0.007	0.280±0.008	**0.330±0.009**	0.262±0.007	0.214±0.006
12.5 mg/kg	0.086±0.002	0.075±0.002	0.092±0.002	0.090±0.002	**0.128±0.003**	0.089±0.002	0.089±0.002

Table.11. Effect of Chromiumon Tolerance index of *Vigna unguiculata* (L.) WALP. On 7th DAS

Chromium Treatments	Phytotoxicity						
	CO-1	CO-2	CO-3	CO-4	CO-7	Vamban-1	Vamban-2
Control	-	-	-	-	-	-	-
2.5 mg/kg	1.980±0.0594	1.722±0.051	1.994±0.059	1.873±0.056	**1.570±0.047**	1.849±0.055	1.983±0.059
5 mg/kg	4.003±0.120	3.890±0.116	4.408±0.132	3.540±0.106	**3.104±0.093**	3.609±0.108	3.663±0.109
7.5 mg/kg	5.982±0.179	6.503±0.195	6.789±0.203	5.528±0.165	**4.842±0.145**	6.440±0.193	5.983±0.179
10 mg/kg	7.490±0.224	8.218±0.246	8.102±0.243	7.112±0.213	**5.910±0.177**	8.325±0.249	8.003±0.240
12.5 mg/kg	8.602±0.258	9.840±0.295	8.952±0.268	8.692±0.260	**6.388±0.191**	9.660±0.289	8.873±0.266

Table.12. Effect of Chromiumon Phytotoxicityof *Vigna unguiculata* (L.) WALP. On 7th DAS

4. DISCUSSION:

However, decline in water absorption and transport along with water stress tolerance (Barcelo et al., 1988) resulting in lower plant growth and development. Moreover, reduction of germination under Cr (VI) stress, is probably due to increase of protease activity and decrease in a- and b- amylase activities (Zeid, 2001; Parmar et al., 2002). The suppression of germination ratewas only recorded at higher Cr concentration. This perhaps due to the disruption in seed coat permeability (Hou et al., 2014).

The reduction in growth of shoot and root might be due to reduction in cell division, deleterious effect of Hg (II) on photosynthesis, respiration and protein synthesis (Vijayaragavan et al., 2011). Moreover, it may be suggested that the suppression in root growth may be due to inhibition of root cell division or elongation, or to the extension of the cell cycle (Ryan et al., 1997).

This decrease indicates that the chlorophyll synthesis system and chlorophyllase activity were affected by the exposure to high chromium concentrations as suggested by Assche and Clijsters, 1990.The present study concluded that the seed and seedling of Cow Pea has potential to counteract the deleterious effects of chromium metal in soil.

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