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EFFECT OF INCREASED SULFUR CONCENTRATION ON THE PHYSIOLOGICAL AND MORPHOLOGICAL RESPONSES OF THE CORIANDER (*CORIANDRUM SATIVUM*) PLANT TO CHROMIUM TOXICITY

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ABSTRACT: *The present study was aimed to investigate the effect of increasing sulfur concentrations in irrigation water in alleviating the adverse effect of chromium toxicity. The cultivated coriander seeds were treated with different concentrations of sulfate (0.4, 0.8 &1.6 mM magnesium sulfate) for 6 weeks until completely growth and ensure for healthy. Each treated group was divided into three subgroups to handle treatment concentration of 0 µM, 50 µM and 100 µM chromium. Stem and root lengths, fresh and dry weights, pigments, protein and chromium concentration were measured. The data showed that increasing chromium concentrations induced a significant decrease in the measured morphological and physiological parameters. Moreover, chromium was accumulated in coriander plant with increasing chromium concentration in irrigation water. Increasing sulfur concentration alleviates the adverse effect of chromium by increasing shoot and root lengths and dry weights. Also, sulfur increased pigment concentration in chromium treated plants to levels as those of control plants. The effect of sulfur was more effective at 50 µM Cr than 100 µM Cr. The two concentration of sulfur were both effective.*

KEYWORDS*: chromium, sulfur, pigments, protein, Cr concentration.*

INTRODUCTION

It is well documented that chromium plays an important role in the metabolism of all living cells. However, at high concentrations it is toxic, mutagenic, and carcinogenic, especially in Cr (VI) form, which causes oxidative stress and DNA damage.Chromium is easily absorbed by the plants from the soil and atmosphere, accumulate in the organs of the plants and show their cytotoxic and phytotoxic effects (Parmar and Chanda, 2005). Accumulation of Cr by plants can reduce growth, induce chlorosis in young leaves, reduce pigment content, alter enzymatic function, damage root cells and cause ultrastructural modifications of the chloroplast and cell membrane (Choundhury and Panda, 2004, Gbaruko and Friday 2007).

Cr shows physiological, biochemical, length, fresh weight, dry weight, and leaf area decrease. This was observed in many plants, such as chickpea (Singh et al., 2010), wheat (Adrees *et al*., 2015; Nayak *et al*., 2015), and *Brassica napus* (Gill *et al*., 2015a). Root growth is frequently affected by chromium. Peralta and coworkers (Peralta *et al*., 2001) showed that 5 mg L^{-1} of Cr (VI) increased root growth comparatively to the control and at higher doses (20 and 40 mg L^{-1}) there was a dose inhibition effect. Cr (VI) in

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concentrations up to 200 mg L^{-1}decreased growth of paddy (*Oriza sativa* L.), (Sundaramoorthy *et al.*, 2010). Moreover, roots of *Zea mays* L. treated with Cr (VI) were shorter, brownish and presented less number of roots hairs (Mallick *et al*., 2010).Stem growth is another parameter usually affected by Cr exposure. Cauliflower grown on sand with 0.5 mM Cr (III) showed suppression of growth and leaves were smaller, chlorotic, and wilted comparatively to control (Chatterjee and Chatterjee, 2000). Also, Dey and coworkers (Dey *et al*., 2009) found decrease in shoot length by 44% comparatively to the control. Concentrations of Cr (VI) in soil of 500 mg kg^{-1} also affected shoot growth of wheat and oat (Lopez-Luna *et al*., 2009). Mallick and coworkers (Mallick et al., 2010) found that shoot length of Zea mays L. decreased significantly at 9 μg mL−1 Cr (VI) after 7 days.

Shakel *et al*, (2017) explored the deleterious effects of different chromium (Cr) stress levels on two maize genotypes. Their results showed that Cr stress decreased the leaf area, cob formation, 100-grain weight, shoot fresh biomass, and yield formation, while Cr accumulation in different maize tissues was found in the order of roots > leaves > stem > seeds in both genotypes. Moreover, the increased Cr toxicity resulted in higher free proline, soluble sugars and total phenolic contents, and lower soluble protein contents. Inhibition of chlorophyll biosynthesis has also been reported in terrestrial plants (Vajpayee *et al*., 2000). Exposure to high level of Cr also causes deleterious effects on plant physiological processes such as photosynthesis, water relations and mineral nutrition. Subsequently, this aggravates the further degradation of pigments, stunting and finally plant death (Gikas and Romanos, 2006; Zou *et al*., 2006).

Cr may affect plant photosynthesis leading to decrease in productivity and ultimately to death. Both Cr (III) and Cr (VI) can cause ultra-structural changes in the chloroplasts leading to inhibition of photosynthesis (Panda and Choudhury, 2005). Moreover, Rodriguez and coworkers (Rodriguez *et al*., 2012) showed that exposure to Cr (VI) induced a reduction of both chloroplast auto fluorescence and volume in pea plants. Cr stress affects the photosynthetic pigment; for example, the total chlorophyll and carotenoid contents were significantly decreased (Adrees *et al*., 2015).Chromium (Cr) can induce degradation of carotenoids in plants (Rai *et al*., 1992). In *Vallisneria spiralis* and other aquatic plants, an increase in carotenoids content was seen under Cr treatment (Vajpayee *et al*., 2001).

Increased concentration of chromium and duration of exposure significantly inhibited the NR activity and protein content of Nymphaea alba (Vajpayee *et al*., 2000). Cr can degrade and hydrolyze proteins and degradation of protein in plants can result in the inhibition of nitrate reductase (NR) activity (Panda and Choudhury, 2004). The crude protein content of both root and shoot in Pumpkin (*Telfairia occidentalis*) decreased significantly with increased in the residual Cr concentrations of the soil. However, higher crude protein content was observed in the shoot than the root (Orhue and Uzu, 2011). The protein content of tree species (*Pithecellobium dulce*, *Tamarindus indicus*, *Pongamia glabra*, *Cassia*

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auriculata and *Azadirachta indica*) is gradually decreased with increase of chromium concentration. (Unnikannan *et al*., 2013).

Kinetic studies of sulfate transport into plant right-side-out purified plasma membrane vesicles have shown that it occurs by sulfate/proton cotransport (Hawsford *et al.* 1993), which agrees with the work on the sulfate uptake system of *Penicillium notatum* (Cuppolett and segel, 1975) and of *Sacchammyces cerwisiae* (Roomans *et al*. 1979). More recent transgenic studies provided evidence that suggests that chromate is absorbed via sulfate transporters in plants.

Apart from the role of sulfate transporters in Cr uptake, S metabolism may affect Cr tolerance and accumulation due to the capacity of certain reduced S compounds to bind and detoxify metals and metalloids (Pilon-Smitset al., 1999; Zhu *et al.*, 1999a, 1999b and Freeman *et al.*, 2004).To investigate the potential role of plant S metabolism in Cr accumulation and tolerance, the interactions between sulfur (S) nutrition and Cr tolerance and accumulation were considered in this study.

MATERIALS AND METHODS

The coriander seeds were obtained from market. Seeds were surface sterilized with 70% ethyl alcohol then washed with water several times. The seeds were tested for germination percentage and it was 98%. Seeds were cultivated in 20 cm in diameter pot half filled with beat moth. Cultivated seeds were divides into 3 groups depending on sulfur concentration (0.4 mM (control), 0.8 mM and 1.6 mM). Each group consists of 45 pots and each pot contains 20 seeds. Seeds were irrigated with Hoagland' solution containing the different concentrations of sulfur for two weeks until suitable growth and ensure for healthy and safety. Each group was subdivided into 3 subgroups and supplied with 0.0, 50 and 100 µM Cr (as potassium dichromate $K_2Cr_2O_7$). Plants were harvested 2, 4 and 6 weeks post chromium treatments.

Measurement of growth and biomass

Growth was measured in terms of shoot and root length along with their dry biomass. Shoot and root length were measured following the harvest in fresh samples. For dry biomass, 2 g of shoot and root tissue for each sample were dried at 80°C for 48h and made complete dry until constant weights achieved. (**Dhindsa *et al*.1981**)

Pigments

Contents of chlorophyll a and b as well as total carotenoids were expressed as mg/g tissue and calculated through the method of (**Metzner *et al*., 1965**). The results were then calculated as mg/g dry weight of leaves.

ISSN 2055-0219(Print), ISSN 2055-0227(online)

Total protein

Protein content was measured according to the method of (**Gornall *et al***., (1949). Total protein = A sample / A standard × 5 g %.

Determination of Chromium in Plant

For the wet digestion a mixture of HNO3/H2O2was used for this procedure, the temperature was maintained at 120°C for 2 h during digestion of 1.0 g of plant sample with 16 mL of 6:2 HNO3/H202 mixtures on the hot plate. After cooling, 10 mL of distilled water was added on the sample and mixed. The residue was filtered through filter paper and then the sample was diluted to 50 mL with distilled water. Metal contents of final solution were determined by ICP-OES. (**Sergio *et al*., 2012**).

Calibrate the instrument for the metals. Establish ICP-OES software run procedures for quantitative analysis. For all sample analyses, the rinse blank used to flush the system between samples

RESULTS

Morphological plant growth:

Shoot and root length (fig. 1&2):

Increasing chromium has a non-significant effect on shoot length all over the experiment period. The data showed that all the sulfur treatments induced increases in shoot length The amount was more pronounced after 6 weeks. Combination between sulfur and chromium significantly increased shoot length after 2 weeks at 1.6 µM S with both 50 and 100 µM Cr and after 4 weeks at 1.6 with 50 µM Cr. Concerning root length, it was found that after 2 and 4 weeks chromium application increased root length when compared to control. The data showed also that all the sulfur treatments induced increases in root length .The amount was more pronounced after 6 weeks. Combination between sulfur and chromium significantly increased root length after 2 weeks at 1.6 µM S with both 50 and 100 µM Cr and after 4& 6 weeks at 1.6 with 100 µM Cr.

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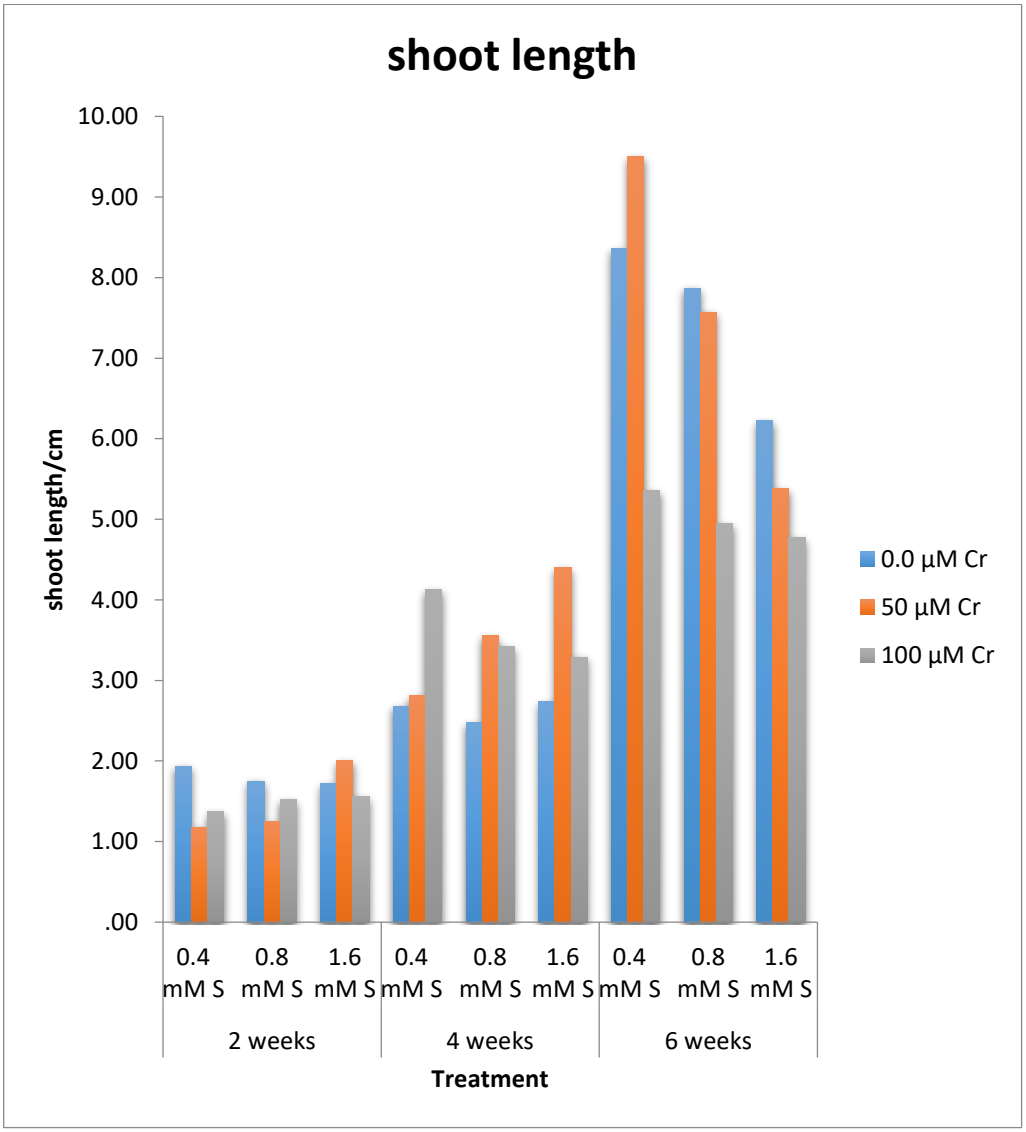

Figure (1): Shoot length(cm) of the Coriander Plant, irrigated with 0.0,50 and 100 µM Cr with or without application of 3 sulfur concentrations 0.4 mM(control) ,0.8 mM and 1.6 mM S.Plants were harvested after 2,4 and 6 weeks. Data presented are mean of 5 replicas ±SE.

ISSN 2055-0219(Print), ISSN 2055-0227(online)

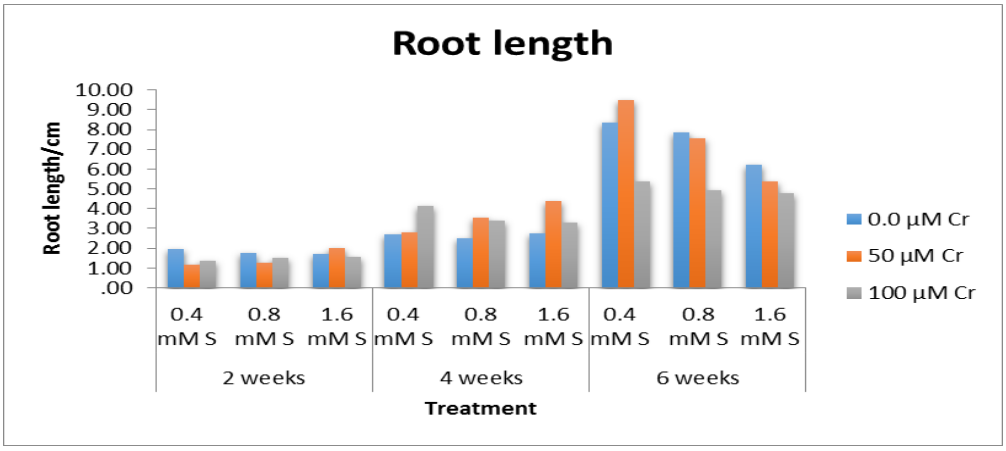

Figure (2): Root length (cm) of the Coriander Plant, irrigated with 0.0,50 and 100 µM Cr with or without application of 3 sulfur concentrations 0.4 mM(control) ,0.8 mM and 1.6 mM S. 4. Plants were harvested after 2,4 and 6 weeks. Data presented are mean of 5 replicas ±SE

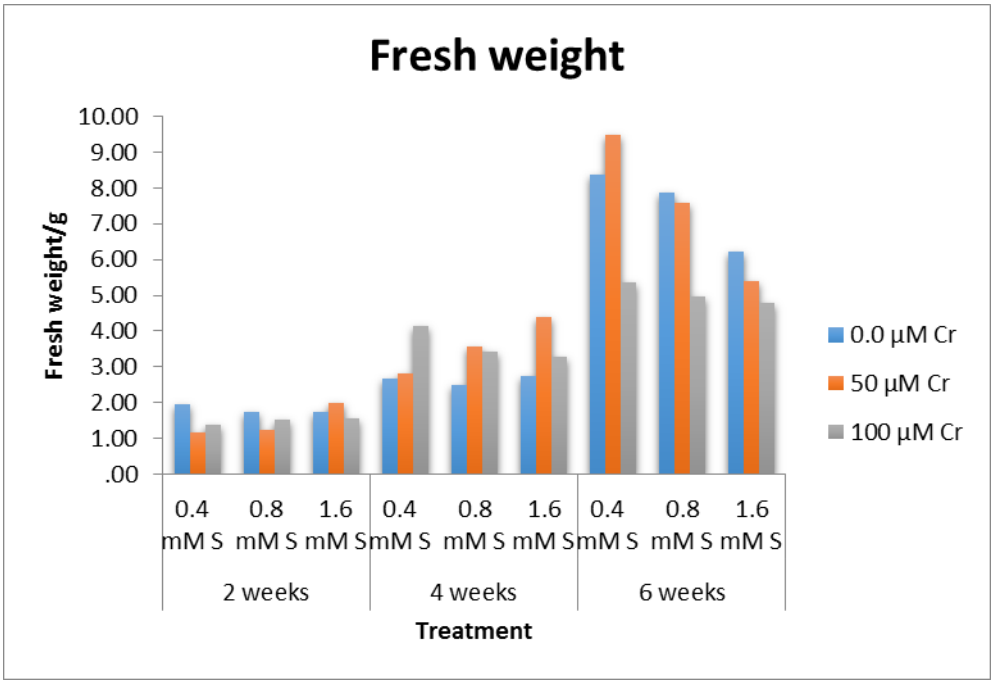

Figure (3): Fresh Weight of the Coriander Plant, irrigated with 0.0,50 and 100 µM Cr with or without application of 3 sulfur concentrations 0.4 mM(control) ,0.8 mM and 1.6 mM S. Plants were harvested after 2,4 and 6 weeks. Data presented are mean of 5 replicas ±SE

ISSN 2055-0219(Print), ISSN 2055-0227(online)

Fresh and dry weights (fig. 3)

Increasing Cr concentration decreased fresh weight of coriander when compared to control at all growth stages. After week 2, Plants in groups treated with1.6 mM sulfur with 0.0 and 50 µM Cr were recognized to have heavier fresh weight than the other experimental groups. Application of 0.8 mM S with 50 & 100 µM Cr significantly increased fresh weight when compared to the same concentrations of Cr alone.

Application of 50& 100 µM Cr with or without S significantly increased dry weight of coriander after 4 weeks of treatments (fig. 4). On the other hand, after 6 weeks, the same concentrations of Cr induced a significant decrease in dry weight when compared to control except at 0.4 mm with 50 Cr µM where the dry weight was increased.

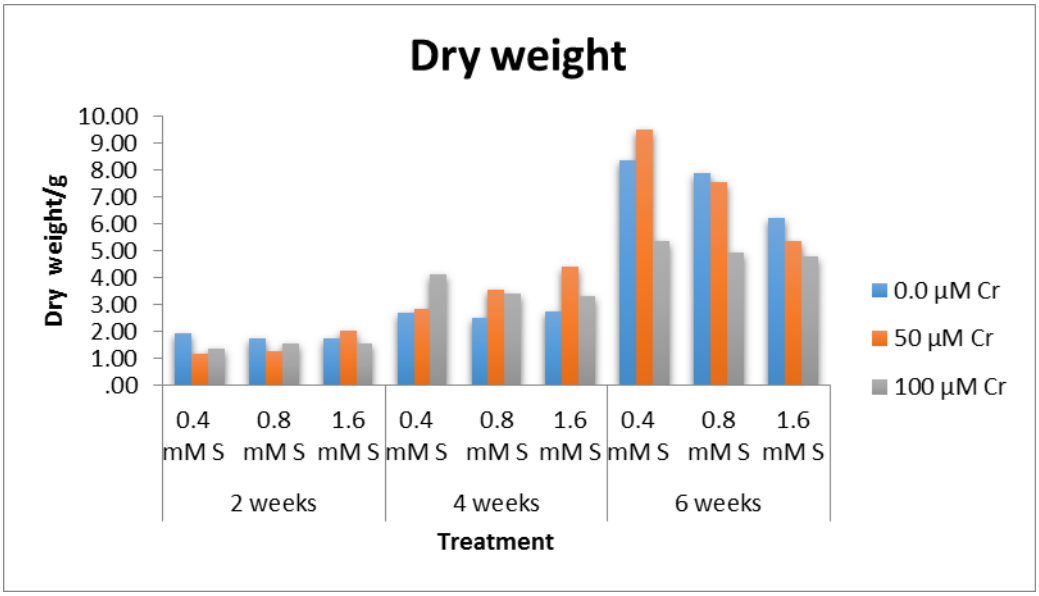

Figure (4): Dry Weight of the Coriander Plant, irrigated with 0.0,50 and 100 µM Cr with or without application of 3 sulfur concentrations 0.4 mM(control) ,0.8 mM and 1.6 mM S. Plants were harvested after 2,4 and 6 weeks. Data presented are mean of 5 replicas ±SE

ISSN 2055-0219(Print), ISSN 2055-0227(online)

Physiological measurements:

Photosynthetic pigments:

Chlorophyll (a):

Data of Chlorophyll a concentration are presented in table (1). Application of Cr induced a significant decrease in Chl.a concentration when compared to control. This decrease was dose dependent. Increasing sulfate concentration (0.8 mM S) increased chlorophyll a concentration in Cr treated plants after 2 & 4 weeks.

Chlorophyll (b), Table 2:

Overall, Chlorophyll b concentration increased with increasing plant age. Chromium treatments significantly decreased Chl. b concentration all over the experimental period. The lowest value was recorded after 6 weeks at 50 µM Cr. Increasing sulfur concentration raised the value of hl. B concentration after 4 weeks at 1.6 mM S with 50 µM Cr and after 6 weeks at 0.8 & 1.6 mM S with 50 and 100 µM Cr.

Carotenoids:

Data of carotenoids is presented in Table (3). The data showed that increasing Cr concentration induced a significant decrease in carotenoid content all over the experimental period. Moreover, increasing sulfur concentration increased carotenoid contents. Treatment of sulfur with chromium alleviates the adverse effect of chromium and induced a significant increase in carotenoid content to the same levels of control.

Table (1): Data of chlorophyll (a) of the Coriander Plant, irrigated with 0.0,50 and 100 µM Cr with or without application of 3 sulfur concentrations 0.4 mM(control) ,0.8 mM and 1.6 mM S. Plants were harvested after 2,4 and 6 weeks. Data presented are mean of 5 replicas ±SE

Plant age	Chlorophyll concentration (mg/g fresh wt) (mean ±SE)			
2 weeks	Treatments	0.0 µM Cr	50 µM Cr	100 µM Cr
	0.4 mM S	2.37±.006	2.11±.067	1.89±.007
	0.8 mM S	2.37±.006	1.96±.017	2.09±.000
	1.6 mM S	1.94±.000	1.92±.010	1.82±.003
F ratio		74.4		
P value		0.000		
4 weeks	0.4 mM S	2.46±.000	2.17±.047	2.13±.000
	0.8 mM S	2.48±.017	2.27±.010	2.33±.003
	1.6 mM S	2.39±.000	2.32±.007	2.35±.003

ISSN 2055-0219(Print), ISSN 2055-0227(online)

F ratio		44.4		
P value		0.000		
6 weeks	0.4 mM S	2.79±.003	2.18±.003	2.40±.003
	0.8 mM S	2.56±.003	2.37±.000	2.29±.047
	1.6 mM S	2.52±.006	2.38±.000	2.19±.007
F ratio		147.4		
P value		0.000		

Table (2) Data of chlorophyll (b) of the Coriander Plant, irrigated with 0.0,50 and 100 µM Cr with or without application of 3 sulfur concentrations 0.4 mM(control) ,0.8 mM and 1.6 mM S. Plants were harvested after 2,4 and 6 weeks.

Plant age	Chlorophyll (b) mg/g fresh wt (mean ±SE)			
2 weeks	Treatments	0.0 µM Cr	50 µM Cr	100 µM Cr
	0.4 mM S	3.06±.003	2.77±.003	2.46±.007
	0.8 mM S	2.67±.000	2.90±.015	2.10±.000
	1.6 mM S	1.74±.000	2.62±.006	2.09±.169
F ratio		58.6		
P value		.000		
4 weeks	0.4 mM S	4.20±.667	3.01±.019	2.93±.007
	0.8 mM S	2.65±.000	2.77±.225	2.73±.165
	1.6 mM S	2.75±.010	3.42±.000	2.57±.003
F ratio		4.6		
P value		.004		
6 weeks	0.4 mM S	4.61±.255	1.30±.023	2.29±.003
	0.8 mM S	2.70±.136	2.05±.007	2.76±.020
	1.6 mM S	2.45±.027	2.49±.000	2.69±.000
F ratio		56.8		
P value		.000		

ISSN 2055-0219(Print), ISSN 2055-0227(online)

Table (3) Data of carotenoids of the Coriander Plant, irrigated with 0.0,50 and 100 µM Cr with or without application of 3 sulfur concentrations 0.4 mM(control) ,0.8 mM and 1.6 mM S. Plants were harvested after 2,4 and 6 weeks. Data presented are mean of 5 replicas ±SE

Plant age	Carotenoids mg/g fresh wt. (mean ±SE)			
2 weeks	Treatments	0.0 µM Cr	50 µM Cr	100 µM Cr
	0.4 mM S	1.45±.000	1.31±.002	1.12±.000
	0.8 mM S	1.52±.000	1.48±.004	1.35±.000
	1.6 mM S	1.75±.000	1.52±.000	1.46±.000
F ratio		5622.7		
P value		.000		
4 weeks	0.4 mM S	1.54±.000	1.59±.005	1.60±.000
	0.8 mM S	1.69±.000	1.58±.000	1.56±.016
	1.6 mM S	1.88±.000	1.59±.000	1.63±.003
F ratio		19.4		
P value		.000		
6 weeks	0.4 mM S	1.68±.057	1.60±.017	1.66±.000
	0.8 mM S	1.91±.003	1.76±.000	1.64±.017
	1.6 mM S	2.12±.000	1.87±.003	1.70±.003
F ratio		20.2		
P value		.000		

Protein content (fig. 5):

All over the experimental period, it was shown that protein content increased with increasing plant age. After 2 and 4 weeks, the data showed a non-significant difference in protein content between Cr treated and untreated plants. After 6 weeks, increasing Cr concentration significantly decreased protein content comparing with control. The lowest value was obtained at 100 uM Cr with 0.8 M Sulfur. Increasing sulfur concentration increased protein content in Cr treated pants. The effect was more pronounced at 1.6 mM S at 100 uM Cr where the % of increase was 17.0%.

The Percentage of Cr in the dry weight (fig. 6):

The percentage of Cr in plant tissues increased with increasing Cr concentration in irrigation solution. Application of sulfur with increasing concentration induced a significant decrease in

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Cr concentration comparing with control. The effect was more pronounced after 6 weeks at 1.6 mM S with 50 µM Cr (46.98% decrease) and with 100 µM Cr (28.16% decrease).

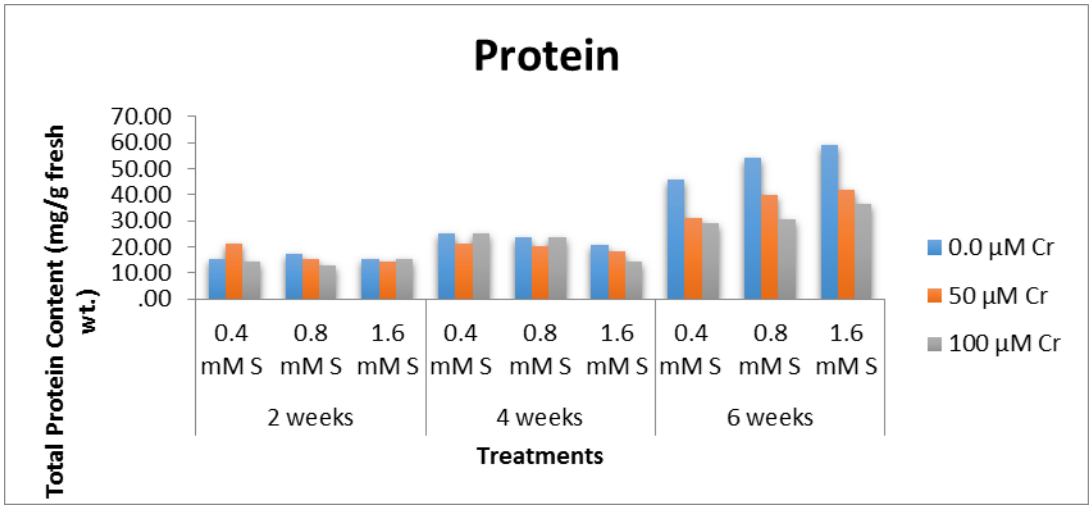

Figure (5) Data of protein of the Coriander Plant, irrigated with 0.0,50 and 100 µM Cr with or without application of 3 sulfur concentrations 0.4 mM(control) ,0.8 mM and 1.6 mM S. Plants were harvested after 2,4 and 6 weeks. Data presented are mean of 5 replicas ±SE

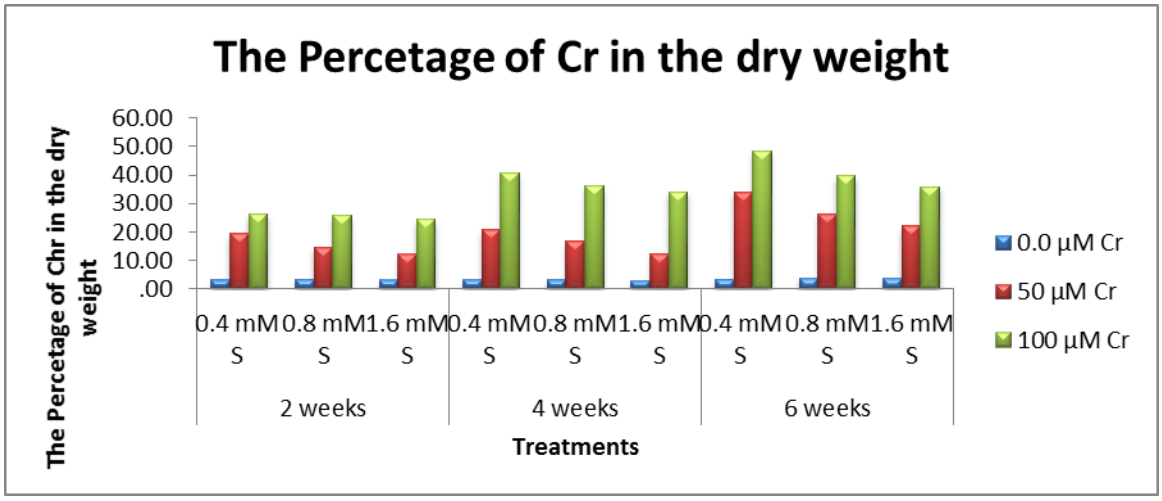

Figure (6) Data of the percentage of Cr in the dry weight of the Coriander Plant, irrigated with 0.0,50 and 100 µM Cr with or without application of 3 sulfur concentrations 0.4 mM(control) ,0.8 mM and 1.6 mM S. Plants were harvested after 2,4 and 6 weeks. Data presented are mean of 5 replicas ±SE

DISCUSSION

Morphological plant growth

Heavy metals like Zn, Fe, Cu and Mn are essential for plant growth and important constituent of many enzymes of metabolic importance. Other metals like Pb, Cd, As, Se Al and Cr are

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biologically non-essential and toxic above certain threshold levels. Cr is toxic to plants and does not play any role in plant metabolism (Dixit et al., 2002). Accumulation of Cr by plants can reduce growth, induce chlorosis in young leaves, reduce pigment content, alter enzymatic function, damage root cells and cause ultrastructural modifications of the chloroplast and cell membrane (Panda, 2003; Choudhury and Panda, 2004 & Hu et al., 2004).

The present study indicated that all level of Cr treatments lead to significant decrease in shoot length and in root length of the Coriander than the control group. Furthermore, the results indicated that 100 µM of Cr treatments lead to significant decrease in fresh weight of the Coriander than the control group. However, 100 µM of Cr treatments lead to significant increase in dry weight of the Coriander than the control group.

These results are in agreement with those reported by (Ruscitti et al. 2011) who reported that moderate or high Cr concentrations reduced all plant growth parameters (roots, stems and leaves dry weight, leaf area and Stem/The total dry weigh ratio). Jamal et al. (2006) demonstrated that Cr produced a significant reduction in the growth of Prosopis juliflora and with (Bishnoi et al. 1993) who determined that the deleterious effect of Cr was more pronounced on the growth of roots than on the stems, this could be due to Cr accumulation in the roots.

Maiti et al. (2012) reported that Cr exposure significantly inhibited plant length and biomass. Moreover, Hamid et al. (2012) reported that decrease in leaf area and curling of leaves may be due to high levels of Cr accumulation in them. This interferes with the function of genes that govern the synthesis of enzymes, which in turn control the chemistry of cell; all this must somehow account for growth and development (Karuppanapandian and Manoharan, 2008; Gheju et al., 2009).

Cr (VI) seems to act principally on plant roots, resulting in intense growth inhibition. Increasing concentration of Cr caused significant reduction in root length and shoot length (Gani, 2011). Cr transport to the aerial part of the plant can have a direct impact on cellular metabolism of shoots, contributing to the reduction in plant height. Cr exposure at micro molar range can lead to serve phototoxic symptoms in plant cell, which can result in inhibition of seed germination, degrade pigments status, nutrient balance and antioxidant enzymes (Panda and Khan, 2003; Panda, 2003). Decreased root growth due to Cr toxicity could be as a result of inhibition of root cell division, root elongation or the extension of cell cycle in root (Chen et al., 2001). The decrease in root growth is a well-documented effect due to heavy metals in trees and crops (Breckle, 1991; Goldbold and Kettner, 1991; Prasad et al., 2001).

The effects of Cr on plant height and shoot growth have been reported earlier by (Rout et al. (1997). Cr transport to the aerial part of the plant can have a direct impact on cellular metabolism of shoots, contributing to the reduction in plant height. Reduced growth in terms of root and shoot lengths at increasing doses of chromium might be due to adverse effect of this metal on auxin synthesis in paddy plants more so during early stages of their growth (Barton et al., 2000). Moreover, oxidative stress caused by Cr or other heavy metals as a severe phytotoxicity in every possible way limit successful plants growth and development.

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Physiological measurements

The present study indicated that all level of Cr treatments lead to numerically decrease in chlorophyll (a) of the Coriander than the control group. Furthermore, the results indicated that 100 µM of Cr treatments lead to significant decrease in chlorophyll (b) and carotenoids of the Coriander than the control group. However, the results indicated that 100 µM of Cr treatments lead to numerically increased in protein of the Coriander than the control group. Also, all level of Cr treatments leads to increase in the percentage of Cr in the dry weight of the Coriander than the control group.

These results are in agreement with those reported by Abdul Razak (1985) who determined a decrease in the photosynthetic activity and in the chlorophyll synthesis by the accumulation of Heavy metals, and by(Schützendübel and Polle, 2002) who reported a significant reduction in proteins content and in enzyme activity by the interference produced of metal ions. The same phenomenon has been observed with exposure to high concentrations of Cr and Al where photo-synthetic pigments (Chl a+b) decreased (Anderson et al., 1973; Karuppanapandian et al., 2006). And with (Rai et al. 1992) who determined that Cr can induce degradation of carotenoids in plants, while chlorophyll content and proteins declined with increasing Cr concentration.

In addition to the reduction in growth and its effect on cell membrane stability, Cr promotes inhibition of photosynthetic pigment synthesis (Vajpayee et al., 2000). Moreover, Hamid et al. (2012) reported that the pigment concentration significantly decreased with the increase in Cr concentration. The chlorophyll pigments are present in thylakoid within chloroplast, and any damage brought to these structures can lead to denaturation of these pigments. It may be suggested that observed decrease in chlorophyll content at higher concentration of chromium may be due to breakdown of thylakoid and chloroplast envelope as was previously reported (Dodge and Law, 1974). There were also significant decreases in chlorophylls a and b content of the B. oleracea var. acephala plants treated with Cr (Ozdener et al., 2011). A decrease in chlorophyll content may either be due to inhibition of chlorophyll synthesis or its destruction or replacement of Mg ions (Barcelo et al., 1985; Chandra et al., 2009). An increase in carotenoid content was observed in Cr treated plants of E. crassipes. Increased carotenoid concentration for the protection from free radical formation is a common response to xenobiotics (Kenneth et al., 2000). Under heavy metal stress condition, rye seedlings showed relatively increasing degradation in the content of chlorophyll a + b in the detached leaves (Krupa et al., 1996). Chlorosis (loss of chlorophyll), which appears in the leaves of Cr-treated seedlings, has been proposed as an indirect effect of Cr, probably due to the retardation of Fe and Zn translocation (Fontes and Cox, 1998).

This general profile of decrease in chlorophyll content at high Cr concentrations suggests that chlorophyll synthesis and/or chlorophyllase activity is being affected. Vajpayee and coworkers (Vajpayee et al., 1999) showed that Cr affects pigment biosynthesis by, for instance degrading α-aminolaevulinic acid dehydrates, an essential enzyme in chlorophyll biosynthesis. Vernay and coworkers (Vernay et al., 2007) also presented evidence that Cr competes with Mg and Fe for assimilation and transport to leaves affecting therefore pigment biosynthesis. As the levels

ISSN 2055-0219(Print), ISSN 2055-0227(online)

of reactive oxygen species (ROS) usually increase as a result of Cr exposure (Pandey et al., 2009), Juarez and coworkers (Juarez et al., 2008) showed that ROS damages pigment-protein complexes located in thylakoid membranes followed by pheophitinization of chlorophylls (substitution of Mg2+ by H+ ions) and destruction of thylakoid membranes.

The decrease in total chlorophyll, chlorophyll a, b and carotenoids have been well documented under Cr stress in plants (Panda and Patra, 1998, 2000; Tripati and Smith, 2000; Panda, 2003; Panda and Khan, 2003; Panda et al., 2003). Cr possesses the capacity to degrade -aminolevulinic acid dehydrates, an important enzyme involved in chlorophyll biosynthesis reactions (Shi and Dalal, 1990). Besides these effects, Cr can alter chloroplast and membrane ultra structure in plants (Choudhury and Panda, 2004). It can cause also ultra structural changes in chloroplast leading to inhibition of photosynthesis. Such alterations in chloroplast have been observed in case of plants like Lemna minor, Pistia species, Taxithelium neplense, and such change in chloroplast membrane structure is accompanied by changes in thylakoid arrangement. Moreover, at high concentration (1 mM), complete distortion of chloroplastic membrane was observed together with severe disarrangement of thylakoids indicating that Cr in hexavalent form can replace many Mg+ ions from active enzymes and cause severe phytotoxic effects (Choudhury and Panda, 2004)

Chromium degrades α-animolevulinic acid dehydratase, which reduces the availability of prophobilinogen required for chl biosynthesis, thereby affecting the amino levulinic acid (ALA) utilization. This causes ALA build up and finally reduces the chlorophyll level (Vajpayee et al., 2001). Decline in chlorophyll content could also be linked to inhibition of biosynthesis of lipids and carotenoids (Palle et al., 1992; Marschner, 1995). Similar observations were reported in Nelumbo nucifera and Spirodela polyrhiza (Vajpayee et al., 1999; Appenroth et al., 2003). The decline in Chl b could be due to destabilization and degradation of proteins of the peripheral part. Higher concentration of Cr caused a breakdown of chlorophyll and an increase in membrane permeability and membrane damage (Vajpayee et al. , 2000; Bertrand and Poirier, 2005; Shanker et al. , 2005).

Chromium toxicity affects plant metabolism, including synthesis of photosynthetic pigments due to metal binding to protein sulphydryl groups (Van Assche and Clijsters, 1990), or by direct destruction of photosynthetic pigments through generation of highly active oxygen radicals (Pinto et al. , 2000). Progressive decline in the photosynthetic rate was observed with application of Cr concentrations. Cr is a strong oxidant with a high redox potential of 1. 38 eV, and may cause serious oxidative damage to the photosynthetic apparatus, as reflected by the results of this study and of (Vernay et al. 2007). The reduced stomatal conductance could be the cause of reduction in photosynthetic rate under Cr treatments.

Increased protein concentration at initial doses of Cr might be due to disturbance in balance of functional part of protein due to excess amount of chromium. Some heavy metals including chromium in excess amount may result into chlorosis which is clearly an effect of iron deficiency in plants. This adverse effect may be caused by change in concentration of essential mineral nutrients. It may also cause reduced photosynthesis resulting from stomatal closure and also reduced intercellular spaces and alteration within chloroplast (Vazquez et al. , 1987). Excess amount of cobalt, chromium and copper had an adverse effect on biomass,

ISSN 2055-0219(Print), ISSN 2055-0227(online)

concentration of iron, chlorophyll "a" and "b", protein and catalase activity in cauliflower (Chatterjee and Chatterjee, 2000).

High levels of Cr were detected in roots of Brassica juncea accumulated more Cr when chromate was offered in the absence of sulfate. This suggests inhibition of chromate uptake and accumulation by sulfate when both anions are present in the growth medium, likely due to competition for the active binding site of the same sulfate transporters. Interaction between sulfate and chromate uptake is also supported by the results obtained from the sulfate uptake experiment, which showed a markedly diminished ability of plants exposed to chromate to take up sulfate from the nutrient solution. (Michela et al., 2008). Concomitant with the reduction in sulfate uptake rates, a significant decrease of transcript accumulation for the low-affinity sulfate transporter BjST1 was observed in plants grown in the presence of Cr under –S and +S conditions. Therefore, other than the probable competition with sulfate for the binding to the same carriers, chromate had an additional way to interfere with sulfate absorption (i.e., the inhibition of low affinity sulfate transporters transcription).

Earlier studies reported a down regulation of BjST1 associated with the activation of the first steps of sulfate reduction and the concomitant enhanced accumulation of thiols in B. juncea in response to cadmium stress (Heiss et al., 1999).

Consequently, the higher sulfate uptake at root level and the enhanced sulfate assimilation were thought to be necessary for providing metal-chelating S compounds, such as GSH and phytochelatins (PCs).

Rather than being a substrate for PCs biosynthesis during Cr stress, GSH may play a major role in the reduction of Cr(VI) to the less toxic trivalent form. In such a reaction, the transfer of three electrons is required, and only few biological compounds in cells function as efficient reductant for Cr(VI), including GSH and ascorbate (Kaim and Schwedersky, 1994).

The oxidation of GSH by Cr(VI) has been previously reported by McAuley and Olatunji (1977). Furthermore, during Cr(VI) to Cr(III) reduction, reactive oxygen species can be produced, and in this view the ascorbate/glutathione cycle might represent a fundamental mechanism regulating the cellular oxidative balance (Noctor and Foyer, 1998).

It could be concluded that Cr induced significant decreases in plant growth as indicated in morphological results. It also decreases pigments and protein content. These adverse effects of chromium were encountered with increasing sulfur supply in the irrigation solution. Although increasing sulfur did not affect the uptake of chromium in coriander plant, but it alleviate most harmful effect of chromium by increasing protein and photosynthetic pigments.

The effect of sulfur was more effective at 50 µM Cr than 100 µM Cr. The two concentration of sulfur were both effective.

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