International Journal of Current Advanced Research

ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 7; Issue 5(I); May 2018; Page No. 12794-12798
DOI: http://dx.doi.org/10.24327/ijcar.2018.12798.2263

Research Article

EFFECT OF HEXAVALENT CHROMIUM ON GERMINATION, SEEDLING GROWTH AND BIOCHEMICAL ALTERATIONS IN *SESAMUM ORIENTALE* L

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A R T I C L E I N F O

Article History:

Received 9th February, 2018
Received in revised form 26th March, 2018 Accepted 17th April, 2018
Published online 28th May, 2018

Key words:

Hexavalent Chromium, Germination, Biochemical changes, Phytotoxicity, Seedling vigour index

A B S T R A C T

Environmental pollution by toxic metal has accelerated dramatically since the beginning of the industrial revolution. The primary source of this pollution include the burning of fossils, mining and smelting of metaliferous ores, municipal waste, fertilizers, pesticide and sewage. Toxic metal contamination of ground water and soil, which poses major environmental and human health problems, is currently in need of an effective and affordable technological solution. An experiment was conducted in Til (*Sesamum orientale* L.) in order to find out the effect of Cr^{+6} toxicity on its germination, growth and biochemical parameters. The seeds were germinated in six different concentrations of Potassium dichromate solution ranging from 0-50 mg/l of hexavalent chromium. It was noted that the Seedling vigour index, Metal tolerance index were found to be reduced and the percentage of phytotoxicity was increased while biochemical parameters showed a declining trend with increasing Cr^{+6} concentrations. The seedlings treated with chromium complexes exhibited decrease in chlorophyll and soluble protein content with the increase in chromium level whereas an increase in proline content was observed as compared to control.

INTRODUCTION

Heavy metals are important environmental pollutants, and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons. The term ‘‘heavy metals’’ refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration. There are 35 metals that concern us because of occupational and residential exposure, out of which 23 are the heavy elements or “heavy metals”. Heavy metal can include elements lighter than carbon and can include some of the heaviest metals (Duffus, 2002). Metals such as aluminium, arsenic, cadmium, cobalt, chromium, copper, lead, manganese, mercury, nickel, selenium and zinc have been considered as the major environmental pollutants and their phytotoxicity has been established (Ross, 1994; Kochian, 1995). It has been reported that metals such as cobalt, copper, chromium, iron, magnesium, manganese, molybedenum, nickel, selenium and zinc are essential nutrients that are required for various biochemical and physiological functions (WHO). Inadequate supply of these micronutrients results in a variety of deficiency diseases or syndromes.

Elevated concentrations of both essential and nonessential heavy metals in soil and water can lead to toxicity symptoms and growth inhibition in most plants (Grant *et al*., 1998; Jindal and Kaur, 2000; Hall, 2002). Absorption, translocation and accumulation of heavy metal ions of Hg, Pb, Cr and Cd by plants, reduce qualitative and quantitative productivity of the species and cause serious health hazards through the food chain to other life forms (Turner, 1994; Barman *et al*., 1999) (Petr *et al*., 1999; Moreno- Caselles *et al*., 2000; Axtell *et al.*, 2003; Cobbett, 2003; Stolt *et al.*, 2006). Different heavy metals at supra-optimal concentrations have been shown to inhibit various metabolic processes in plants resulting in their reduced growth and development (Bala and Setia, 1990; Davies *et al*., 1991; Bernier *et al.*, 1993; Lang *et al.*, 1995;) (Shaw, 1995; Tomar *et al.*, 2000).

Chromium is one of the toxic heavy metals, and is recognized for its negative effects on the environment where it bio-accumulates and poses a serious threat to environmental health. Chromium toxicity affects the plant growth and metabolism to a considerable extent, which includes stunted growth, chlorosis, reduced crop yield, delayed germination, senescence, premature leaf fall, biochemical lesions, enzymatic changes and reduced biosynthesis (Panda and Patra, 1997a and b; Panda and Patra 1998; Zayed *et al*., 1998; Srivastava *et al*., 1999; Zayed and Terry 2003; Mohanty *et al*., 2005 a; Mohanty *et al*., 2009; Mohanty *et al*., 2010 a and b).

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Chromium exists in two stable states, i.e. hexavalent chromium (Cr^{+6}) and trivalent chromium (Cr^{+3}) of which Cr^{+6} is the most toxic form. Cr^{+3} is essential for animal and human health (Katz and Salem, 1994; Mohanty and Patra, 2011) but unlike Cr^{+3}, Cr^{+6} is a potent, extremely toxic, carcinogenic and causes death to animals and humans, if ingested in large doses (Zayed and Terry, 2003; Mohanty and Patra, 2011). Open cast chromite mining activity leads to various environmental problems due to released Cr^{+6}. Contamination of soil and water in chromite mining areas is a serious environmental problem (ATSDR 1998; USEPA 1998).

The use of green plants to remove pollutants from the environment or to render them harmless is phytoremediation (Salt *et al.*, 1998). This technique is a cost effective plant based approach for removal of heavy metals from soil (Chaney *et al.*, 1997; Jena *et al.*, 2004; Ghosh and Singh, 2005) and water (Terry and Banuelos, 2000; Mohanty *et al.*, 2005b; Mohanty and Patra 2011). As Chromium (Cr^{+6}) is an important heavy metal pollutant, the present study was undertaken with an objective to determine its effect on the growth and development of *Sesamum orientale* L.

MATERIALS AND METHODS

Selection of plant material

Sesamum orientale L. belongs to an angiospermic family Pedaliaceae and commonly called Til/Sesame is one of the most ancient oil seed crops known to mankind. Sesame plays an important role in human nutrition. Most of the sesame seeds are used for oil extraction and the rest are used for edible purposes (El Khier *et al.*, 2008). Seeds of *Sesamum orientale* L. were obtained from "National Seed Corporation", Bhubaneswar and seeds stored in dark and cool place for experimental use.

Experimental Design

The present study was undertaken with hexavalent chromium (Potassium dichromate) at 10, 20, 30, 40 and 50 ppm along with control (untreated). Twenty seeds of *Sesamum orientale* L. were each of were surface sterilized with 0.1% mercuric chloride and washed thoroughly with tap water and then with distilled water. Twenty uniform sized seeds were placed in petri-dishes of 10 cm diameter with different concentrations of Potassium dichromate solution (10, 20, 30, 40 and 50 mg Cr^{+6}/l) and one with control at a constant temperature of 26 °C. The seeds were submerged in 10 ml of Hoagland's nutrient solution containing different concentrations of Cr^{+6} twice a day. Each treatment was replicated five times. The number of seeds germinated in each treatment was counted on 5th days of after sowing and the total germination percentage was calculated. Tolerance index (Turner *et al.*, 1994) and Vigour index (Abdul-Baki *et al.*, 1973) of seedlings were calculated.

In pot culture experiment seeds were sown in pretreated soil (soil treated with 10, 20, 30, 40 and 50 ppm Cr^{+6}/kg) and different growth as well as biochemical parameters like total chlorophyll content (Arnon, 1949), total protein content (Lowery *et al.*, 1951) and free proline content (Bates *et al.*, 1973) were estimated in plants at 30, 45 and 60 days after treatment.

This experiment was done in triplicates and the data was statistically analyzed and standard errors of mean (SEM) was calculated.

RESULTS AND DISCUSSION

Germination study

Significant changes were found in the germination of *Sesamum orientale* in different concentration of hexavalent chromium. The germination percentage was decreased with increased concentration of Cr^{+6}.

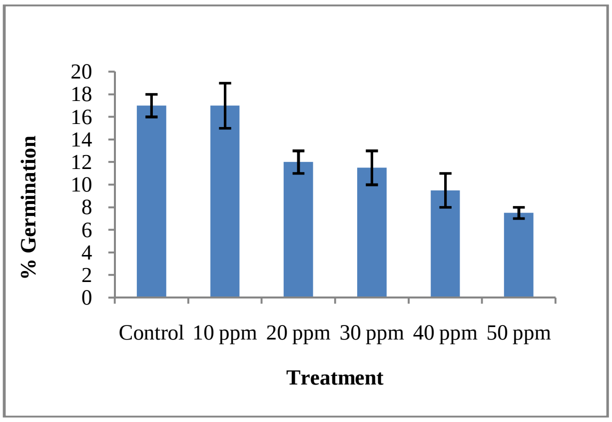

Figure1 Effect of different levels of Cr^{+6} on germination of *Sesamum orientale* seeds.

Observations on the germination study of *Sesamum orientale* L., depicted in Table 1 and Fig 1 clearly indicated that the Seedling vigour index and Metal tolerance index were decreased with increase in concentration of Cr^{+6} while the phytotoxicity was increased with increase in Cr^{+6} concentration. The significant decrease in radicle length of *Sessamum orientale* L. seedling suggests that low concentration of chromium was beneficial for seed germination.

Analysis of Biochemical parameters

Effects on Chlorophyll content of Sesamum orientale **L.**

The effect of varied concentrations (10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm) of Cr^{+6} with untreated soil on chlorophyll synthesis in *Sesamum orientale* L. seedlings have been depicted in Table 2.

Table 1 Effect of Cr^{+6} on seed germination of *Sesamum orientale* L.

Concentrations of Cr^{+6}	Germination (%)	Radicle length (cm)	Standard Vigour Index	Metal Tolerance Index	Pytotoxicity (%)
Control (0.0 ppm)	85±1	8.4±0.3	714	100	0
10 ppm	85±2	8.4±0.2	714	100	0
20 ppm	60±1	7.55±0.25	453	89	10.11
30 ppm	57.5±1.5	7.05±0.05	405.37	83.92	16.07
40 ppm	47.5±1.5	6.55±0.05	311.12	77.97	22.04
50 ppm	37.5±0.5	5.56±0.25	211.87	67.26	32.73

*Values of 5 replicate ±SD

Table 2 Effect of Cr^{+6} on total chlorophyll content of 30, 45 and 60 days old *Sesamum orientale* L. plant

Conc. of Cr^{+6}	**Total Chlorophyll (mg/g fresh wt.)**		
	Days of Treatment		
	30 DAT	**45 DAT**	**60 DAT**
Control (0.0 ppm)	2. 4196±0.0084	2.5605±0.0085	2.719±0.0082
10 ppm	2.1768±0.0082	2.186±0.008	2.2479±0.08
20 ppm	2.0649±0.0079	2.0979±0.0071	2.1136±0.0077
30 ppm	1.5318±0.0068	1.696±0.0057	1.9754±0.0069
40 ppm	1.2828±0.005	1.4044±0.0046	1.788±0.00696
50 ppm	1.1332±0.0046	1.212±0.0041	1.4429±0.0053

*Values of 3 replicates±SEM; DAT: Days after treatment

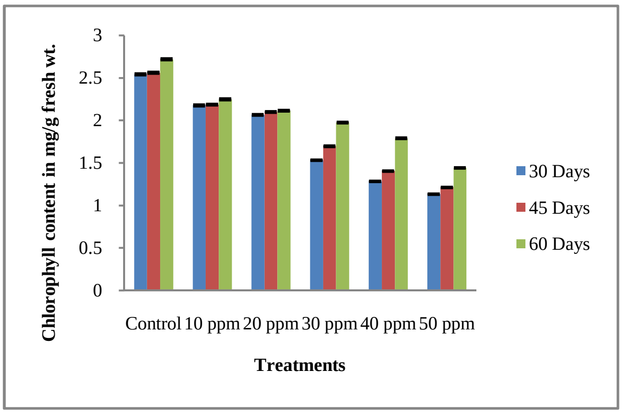

Figure 2 Effect of different levels of Cr^{+6} on the Chlorophyll content in *Sesamum orientale* L. after 30, 45 and 60 days of treatment.

A gradual decease in chlorophyll content in *Sesamum Orientale* L. was observed with the increase in the levels of Cr^{+6} from 0 ppm to 50 ppm irrespective of the period of exposure (i.e. 30 days, 45 days and 60 days).

Effect of Cr^{+6} on Soluble protein content of Sesamum orientale L.

There was gradual decrease in protein content with rise in different levels of Cr^{+6} concentrations from 0 ppm to 50 ppm (Table 3).

Table 3 Effect of Cr^{+6} on soluble protein content of 30, 45 and 60 days old *Sesamum orientale* L.plant.

Conc. of Cr^{+6}	**Protein content (in mg/g fresh wt.)**		
	30 DAT	**45 DAT**	**60 DAT**
Control (0.0 ppm)	2.138±0.237	2.519±0.133	4.366±0.198
10 ppm	2.034±0.005	2.128±0.109	4.326±0.079
20 ppm	1.173±0.163	2.064±0.064	3.969±0.445
30 ppm	1.851±0.091	1.866±0.024	3.569±0.143
40 ppm	1.064±0.054	1.796±0.034	3.544±0.99
50 ppm	0.999±0.029	1.663±0.109	2.91±0.227

*Values of 3 replicate ±SEM; DAT: Day after treatment

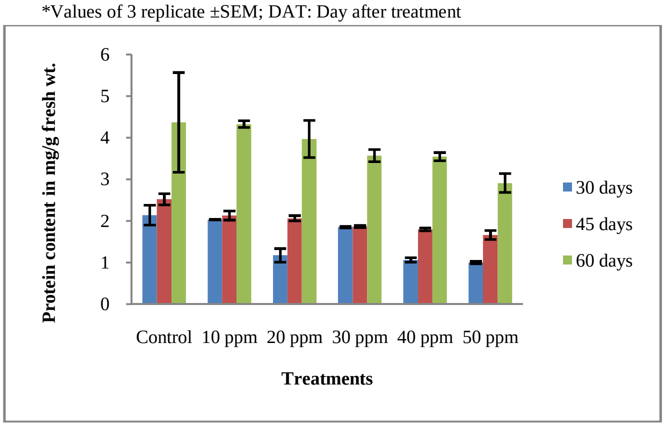

Figure 3 Changes in soluble protein content in *Sesamum orientale* L. after 30, 45 and 60 days of treatment

Protein is produced in less amount when plants are subjected to environmental stress. In the present study the protein content of *Sesamum orientale* L. was found unaffected up to 10 ppm Cr^{+6}concentration treatment. However, a gradual decrease in protein content was observed with the increase in Cr^{+6} level from 20 ppm to 50 ppm irrespective of the period of exposure of the plant to Cr^{+6}.

Effect of hexavalent Chromium on Proline content of Sesamum orientale L

Proline is produced in high amounts when plants are subjected to environmental stress. There was a gradual increase in proline content in the test plant with rise in levels of Cr^{+6} from 0 ppm to 50 ppm which might be due to Cr^{+6} stress (Table 4; Fig 4).

Table 4 Effect of Cr^{+6} on proline content of 30, 45 and 60 days old *Sesamum orientale* L. *plant*

Conc. of Cr^{+6}	**Proline content (in µg/g fresh wt.)**		
	30 DAT	**45 DAT**	**60 DAT**
Control (0.0 ppm)	1.751±0.404	5.119±0.763	0.134±0.445
10 ppm	2.649±0.045	10.484±1.728	3.659±0.112
20 ppm	3.637±0.718	11.853±3.053	3.839±0.157
30 ppm	5.679±1.324	12.055±1.145	5.0967±0.157
40 ppm	9.249±2.739	13.694±2.649	7.498±2.828
50 ppm	11.202±0.696	14.098±3.277	8.171±4.624

*Values of 3 replicate ±SEM; DAT: Day after treatment

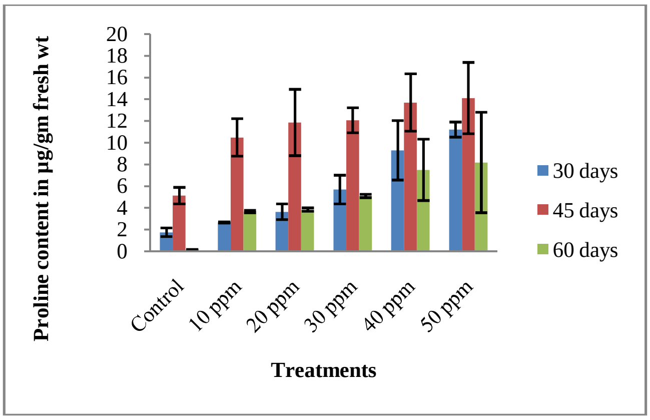

Figure 4 Effect of different levels of Cr^{+6} on the Proline content after 30, 45 and 60 days of treatment

The possible cause might be due to the stimulation of some of the enzymes of the proline biosynthetic pathways by Cr^{+6} which caused the pronounced synthesis of proline at different level of Cr^{+6} concentrations. Maximum proline content was observed in seedling treated with 50 ppm of Cr^{+6} and minimum in control irrespective of the days of treatments. Interestingly the proline content was observed to be highest after 45 days of treatment at all the concentration of hexavalent chromium.

Now-a-days heavy metal stress is considered as one of the emerging type of stresses. This is caused due to various kinds of natural as well as man-made activities. Hauschild (1993) reported that hexavalent chromium (Cr^{+6}) was more toxic than trivalent (Cr^{+3}) and thereby causing severe disturbances in the biological systems.

CONCLUSION

Toxicity of heavy metals has received considerable attention partly due to its occurrence in nature and by mining activities. The data on growth parameter study showed that, with the increase in Cr concentration, the growth rate decreased progressively. Chromium (Cr^{+6}) at higher levels may inhibit the growth and development directly by inhibition of cell division or cell elongation or combination of both, resulting in the limited uptake and translocation of nutrients as well as water which causes mineral deficiency. At higher concentrations it acts as a toxic metal. From the result of this investigation, it can be concluded that hexavalent chromium at lower concentration has a stimulating effect on the germination process and seedling growth and will inhibit the same at higher concentrations.

The overall study demonstrated that among all six types of treatment, control showed high chlorophyll content as well as similar result found in protein content. This is due to the fact that, the seedlings exhibited stunted growth for which unusual metabolism was noticed.

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How to cite this article:

Pallavi Sahoo *et al* (2018) 'Effect of Hexavalent Chromium on Germination, Seedling Growth And Biochemical Alterations in Sesamum Orientale L', *International Journal of Current Advanced Research*, 07(5), pp. 12794-12798.
DOI: http://dx.doi.org/10.24327/ijcar.2018.12798.2263

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