Morphological and physiological changes in snake gourd (*Trichosanthes cucumerina* L.) under chromium stress

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Abstract

Background: The aim of the present study is to assess various morphological and physiological parameters to explore the effect of different levels of heavy metal chromium (0.05, 0.1, 0.15, 0.2 and 0.25 g/kg soil) in *Trichosanthes cucumerina* L. seedlings. **Methods:** Various morphological (shoot length, root length, number of leaves, leaf area, fresh weight, dry weight and tolerance index percentage) and physiological (chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid content). **Findings:** The plant growth characteristics such as shoot length, root length, number of leaves, leaf area, fresh weight, dry weight and tolerance index were adversely influenced by application of chromium in *T. cucumerina* seedlings. Likewise, the toxicity induced by Cr was resulted in significant decrease in contents of photosynthetic pigments chlorophyll and carotenoids.

Key words: Chromium, Heavy metal, Reactive oxygen species, Stress.

1. Introduction

Toxic metal induced by environmental pollution has dramatically increased because of various human activities during and after industrial revolution. Various human activities promote the enrichment of world-wide agricultural soil with a myriad of chemical pollutants. Soil contamination, due to heavy metals such as mercury (Hg), cadmium (Cd), lead (Pb), chromium (Cr), and arsenic (As) was a major environmental challenge worldwide [1].

Chromium is a non-essential element for plants and is known to be a toxic metal that can cause severe damage to plant growth and development. Discharge of sewage and industrial effluent into surface water bodies directly or indirectly cause accumulation of Cr and other toxic metals in soil and causing toxicity to plants [2]. Cr induced oxidative stress involves inhibition of seed germination and induction of lipid peroxidation in plants that causes severe damage to the morphological, physiological and biochemical properties of plants. Oxidative stress induced by Cr initiates the degradation of photosynthetic pigments causing decline in growth. Cr in high concentration can disturb the chloroplast ultrastructure there by disturbing the photosynthetic process. Cr can affect antioxidant metabolism in plants. Various studies reported that Cr toxicity in plants triggers the production of reactive oxygen species (ROS) and lipid peroxidation. These overexpressed ROS can desynchronize the antioxidant defence systems (enzymatic and non-enzymatic) besides malformation of chloroplast ultrastructure, and degradation of photosynthetic carbon assimilation mechanism that ultimately leads to the reduction in plant growth and biomass [3]. In this study, a pot experiment was conducted using snake guard plant (*Trichosanthes cucumerina* L.) grown in Cr polluted soil conditions to assess various morphological and physiological parameters to explore the effect of different levels of Cr toxicity in *Trichosanthes cucumerina* L.

2. Materials and Methods

2.1 Experimental area

The experiment was carried out at botanical garden K. A. H. M. Unity Women's College, Manjeri, Malappuram, Kerala from January – June 2022 under natural conditions. The climate is generally hot and humid. The range of temperature varying between 32 and 28 ^{0}C. The average rainfall is 290 mm.

2.2 **Plant material**

Trichosanthes cucumerina L. commonly known as snake gourd. Seeds of Manusree variety were collected from Regional Agricultural Research Station (RARS) of Kerala Agricultural University, Pattambi, Kerala, India.

2.3 **Experimental setup**

The pot experiment was conducted under the green house conditions for 6 months. Snake gourd was selected as experimental crop. 3-5 seeds were grown in plastic grow bags containing soil, weighing about 4 Kg. After germination, the seedlings were thinned and these with best growth performance were retained (3 plants per pot). Irrigation was done regularly using tap water.

2.4 Chromium treatment

After 21 days of sowing, the soil was treated with Potassium dichromate ($K_2Cr_2O_7$) solution of different concentrations (0.05, 0.1, 0.15, 0.2 and 0.25 g/kg soil) along with an untreated control. The experiment was conducted in triplicates. After Chromium treatment all pots including control were equally watered measuring 200 ml of tap water. The growth of plants was monitored daily to fix the day of stress showing severe conditions in plants. On the second day, toxic limits of chromium were found in plants treated with 0.15, 0.2 and 0.25 g/kg soil. In the same day all treated plants including control were separated out from the experiments for analysis. The collected plant samples were washed with running tap water to get rid of soil or contaminants at the root zone and kept immediately in to plastic bags and the samples were refrigerated for analyzing various morphological and physiological parameters.

2.5 Morphological studies

The shoot length, root length, number of leaves, leaf area, fresh weight and dry weight were measured. Tolerance index percentage (TI) is calculated using root length of control and treatments.

$$TI = \frac{observed\ value\ of\ root\ length\ in\ solution\ with\ metal}{observed\ value\ of\ root\ length\ in\ solution\ without\ metal} \text{ X } 100$$

2.6 Physiological parameters

The total chlorophyll and carotenoid content in leaves were estimated following the method on Arnon (1949) [4] using 80% acetone was used as the extracting medium.

2.7 **Statistical analysis**

The data is an average of recordings from three independent experiments each with three replicates (*i.e.* n=9). The data represent mean±standard error (S.E.).

3. Results

The present study has been aimed to investigate the toxic effects of different concentrations of chromium stress induced *Trichosanthes cucumerina* L. seedlings and evaluated the various morphological and physiological parameters.

3.1 Morphological parameters

Shoot length of Cr (0.05, 0.1, 0.15, 0.2, 0.25 g/kg soil) treated snake gourd plants were significantly decreased by Cr treatment compared with the untreated plants. Compared to control plants, maximum decrease of shoot length was clearly visible in the plants treated with 0.25g (26%) as compared to other treatments of Cr. However, the percentage of decrease in shoot length of plants treated with 0.05g was less (11%) as compared to control seedlings than other stress treatments. Moreover, shoot length of the plants subjected to 0.1, 0.15 and 0.2 g Cr stress also decreased as compared to control snake gourd plants (16, 18 and 20% respectively). The root length was also decreased with increasing Cr concentration in snake gourd plants and maximum decrease (20%) was observed when subjected to 0.25 g Cr as compared to control plants. Likewise, the declines in root length were found by 4%, 10% and 14% in snake gourd plants subjected to 0.1, 0.15 and 0.2 g Cr stress, respectively as compared to control seedlings. However, there was only 3% variation in snake gourd plants treated with 0.05 g Cr as compared to untreated plants (Table 1; Fig. 1).

Number of leaves per plant was counted to analyze the morphological variations of different Cr treatments in snake gourd seedlings. In the case of snake gourd plants subjected to 0.2 and 0.25 g/kg soil Cr, the number of leaves per plant was slightly reduced (20%) as compared to control plants. However, there is no noticeable reduction on the number of leaves per snake gourd plants after subjected to 0.05, 0.1 and 0.15 g/kg soil Cr. With increase in the Cr stress concentration (0.05-0.25 g) in the soil induced a decline in the leaf area of snake gourd plants and the deleterious effect of Cr became more severe with increasing Cr level. The reduction in leaf area was less in snake gourd plants subjected to 0.05 and 0.1 g Cr stress (9 and 11% respectively) as compared to control plants and the decline in leaf area was maximum in plants subjected to 0.2 and 0.25 g Cr (21 and 27%) as compared to control plants. Moreover, snake gourd plants treated with 0.15 g Cr showed 14% reduction in leaf area as compared to control plants (Table 1; Fig. 1).

Snake gourd plants subjected to 0.05g and 0.1g Cr stress showed 19% and 23% reduction in fresh weight as compared to control plants. Likewise, fresh weight of snake gourd plants subjected to 0.2 and 0.25 g Cr was highly decreased (37 and 54%, respectively) as compared to untreated plants. Compared to control plants, maximum decrease of dry weight was recorded in the plants treated with 0.2 and 0.25 g Cr (29 and 36%, respectively) as compared to other treatments. Whereas, other treatments of Cr induced reduction in dry weight was less as compared to untreated snake gourd plants. There were significant differences in tolerance index (TI) in snake gourd plants exposed to different Cr concentrations. Compared with the control plants, the TI changed little when snake gourd plants were treated with 0.05 and 0.1 g/kg soil Cr, while TI was decreased obviously after 0.15, 0.2 and 0.25 g Cr treatments (10, 14 and 20%, respectively) as compared to untreated snake gourd plants (Table 1).

Table 1: Shoot length (cm), root length (cm), number of leaves, leaf area (cm^2), fresh weight (g) and tolerance index (%) of snake gourd plants treated with different concentrations of chromium (0, 0.05, 0.1, 0.15, 0.2 and 0.25 g/kg). Values given are mean of 3 independent experiments, each with a minimum of 3 replicates (i.e. n=9) ± S.E.

Cr treatments (g/kg soil)	Shoot length (cm)	Root length (cm)	Number of leaves	Leaf area (cm^2)	Fresh weight (g)	Dry weight (g)	Tolerance Index (%)
Control	27.9±1.93	42.3±2.43	5±0.29	25.4±1.67	7.68±0.32	0.93±0.04	100±6.23
0.05	24.7±2.12	41±1.62	5±0.87	22.9±2.06	6.19±0.29	0.85±0.02	96.93±4.03
0.1	23.3±1.96	40.5±1.57	5±0.34	22.4±1.76	5.87±0.21	0.68±0.04	95.74±6.06
0.15	22.6±2.27	37.9±1.13	5±0.64	21.8±1.46	4.94±0.19	0.67±0.05	89.52±4.86
0.2	22.1±1.74	36.1±2.17	4±0.16	19.8±1.93	4.79±0.21	0.65±0.03	85.37±6.35
0.25	20.4±2.37	33.5±1.66	4±0.26	18.3±2.16	3.46±0.18	0.59±0.02	79.16±3.51

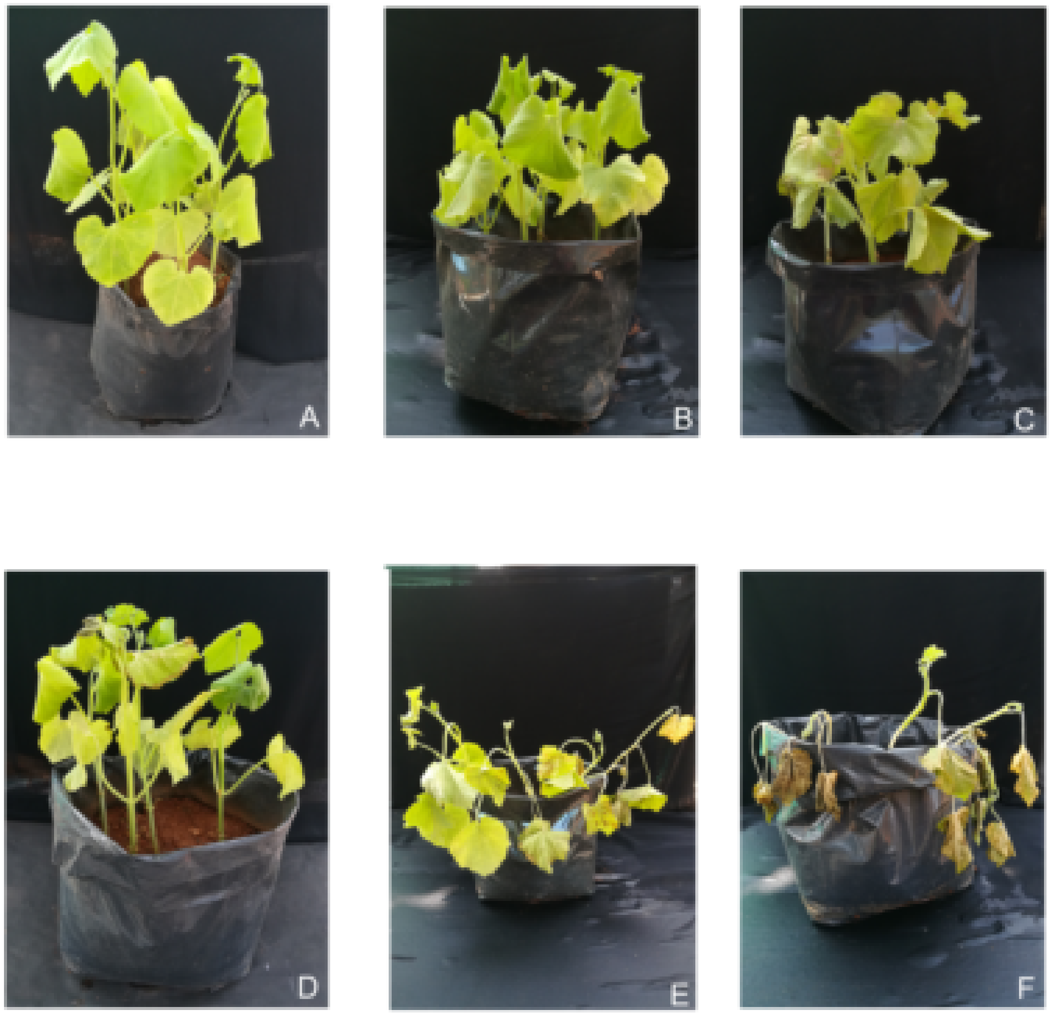

Figure 1: Effects of different concentrations of chromium (0.05, 0.1, 0.15, 0.2 and 0.25 g/kg soil) in snake gourd seedlings. A-Control, B-0.05 g/kg soil, C-0.1 g/kg soil, D-0.15 g/kg soil, E-0.2 g/kg soil, F-0.25 g/kg soil.

3.2 Physiological parameters

Chlorophyll *a*, *b* and total chlorophyll content in snake gourd plants exposed to various concentrations of Cr was decreased as compared to control leaves. The chlorophyll *a* content was declined with increasing Cr concentration in snake gourd plants and maximum decrease (90%) was observed when subjected to 0.25 g Cr as compared to control plants. Snake gourd plants treated with 0.1 and 0.15 g Cr showed 60 and 72% reduction in chlorophyll *a* content as compared to control plants. Likewise, the declines in chlorophyll *b* content were found by 13, 25 and 45% in snake gourd plants subjected to 0.05, 0.1 and 0.15 g Cr stress, respectively as compared to control seedlings and the decline in chlorophyll b content was maximum in plants subjected to 0.2 and 0.25 g Cr (70 and 82%) as compared to untreated leaves. Moreover, total chlorophyll content was also reduced by various concentrations of Cr treatments (0.05, 0.1, 0.15, 0.2 and 0.25 g/kg soil) and maximum reduction was recorded after exposure to 0.2 and 0.25 g/kg soil Cr (76%) as compared to untreated snake gourd plants (Fig. 2).

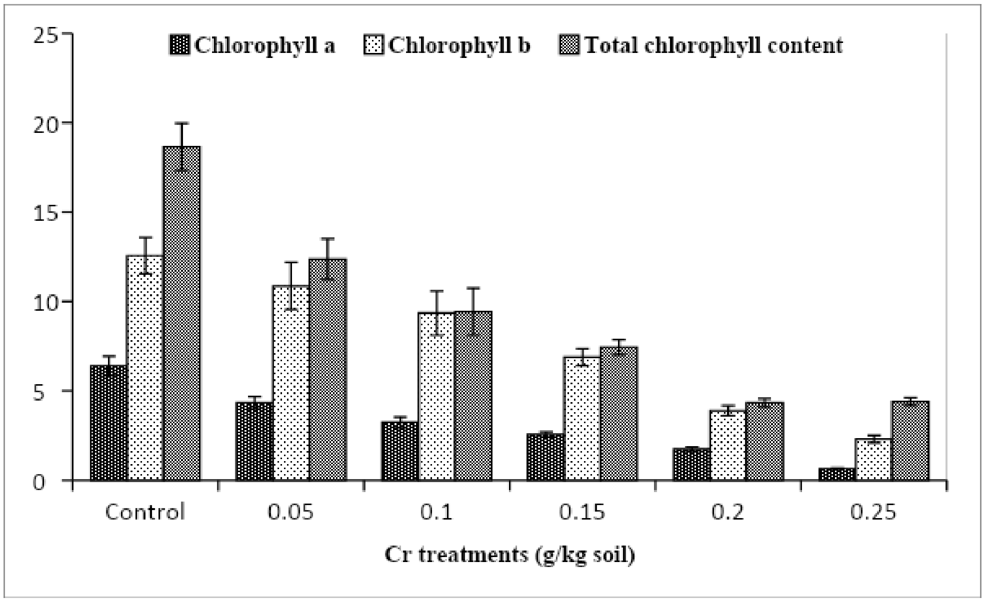

Figure 2: Chlorophyll a, b and total chlorophyll content (mg/g DW) of snake gourd plants treated with different concentrations of chromium (0, 0.05, 0.1, 0.15, 0.2 and 0.25 g/kg). The vertical bars represent S.E. of the mean value of recordings from 3 independent experiments each with a minimum of 3 replicates (i.e. n=9).

The carotenoid content in leaves was also decreased in snake gourd plants subjected to 0.05-0.25 g Cr stress and the maximum reduction (80-83%) was found when treated with 0.2 and 0.25 g Cr. The declines in carotenoid content were found by 45% in snake gourd plants subjected to 0.15 g Cr stress as compared to control seedlings. However, the reduction in carotenoid content was less upon exposure to 0.05 and 0.1 g Cr stress as compared to control plants (Fig. 3).

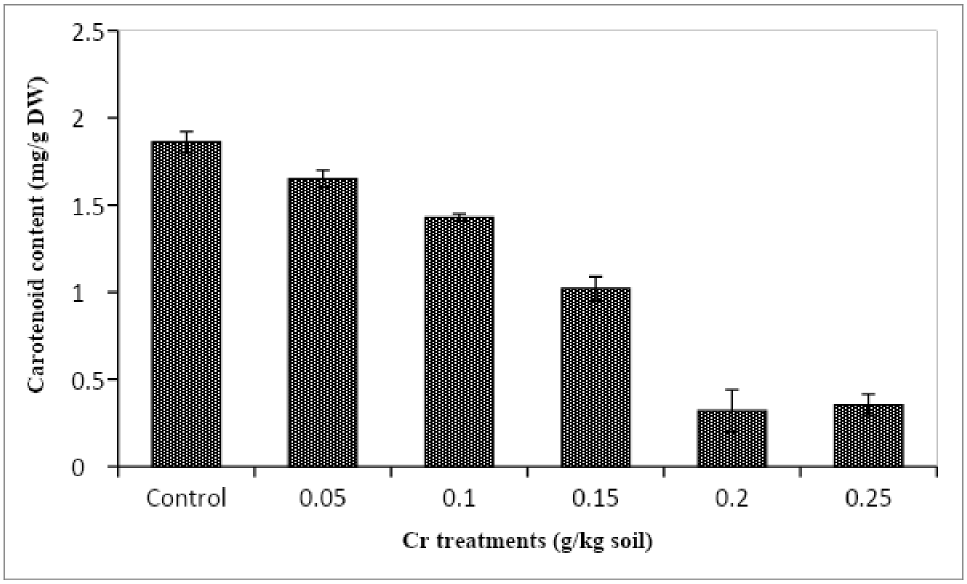

Figure 3: Carotenoid content (mg/g DW) of snake gourd plants treated with different concentrations of chromium (0, 0.05, 0.1, 0.15, 0.2 and 0.25 g/kg). The vertical bars represent S.E. of the mean value of recordings from 3 independent experiments each with a minimum of 3 replicates (i.e. n=9).

4. Discussion

Various morphological and physiological parameters to analyze the toxic effects of different concentrations of Cr (0.5-0.25g/kg soil) in *T. cucumerina*, were recorded. The results showed that the plant growth characteristics such as shoot length, root length, number of leaves, leaf area, fresh weight, dry weight and tolerance index were adversely influenced by application of Cr in different concentrations (0.05, 0.1, 0.15, 0.2 and 0.25 g/kg soil) in 21 days old snake gourd seedlings. However, the decrease in these morphological parameters of snake gourd plants treated with 0.05 g/kg soil Cr was less lethal than other stress treatments. The growth parameters of the plants subjected to 0.1, 0.15 and 0.2 g/kg soil Cr was significantly decreased.

Chromium stress imposition also resulted in decreased root and shoot length of radish seedlings at 1.5 mM concentration [5] and the growth inhibition by Cr can be due to chromosomal aberrations which lead to inhibition of cell division [6]. Previously reports states that the reduction in shoot length might be due to Cr induced ultrastructural damages to leaf mesophyll cells [7,8]. Similarly the morphological traits of cauliflower under Cr stress, plant height, root length, number of leaves, leaf area and dry weight were decreased as compared to control plants [14]. The reduction in growth and biomass might be attributed to the Cr interaction with essential nutrients and reduced their uptake by plants [10].

Moreover, there were significant differences in tolerance index also observed in snake gourd plants exposed to different Cr concentrations. Little change in TI was recorded in snake gourd plants treated with low concentrations (0.05 and 0.1 g) of Cr. However, tolerance index is decreased in the higher concentrations of Cr treated snake gourd plants as compared to control plants. Likewise, the toxicity induced by Cr resulted in significant decrease in contents of total chlorophyll, Chlorophyll *a* and chlorophyll *b.* About 1.5 times decrease in total chlorophyll content was decreased in 1.5 Mm Cr stress than control in *Raphanus sativus* [5]. Similarly, Cr stress significantly decreased the photosynthetic pigments including chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids as compared to control plants in cauliflower plants [11]. The decrease in photosynthetic pigments might be due to ultrastructural changes in chloroplast [7] or production of ROS under Cr stress [9]. Collectively, this study provided an insight into the various morphological, physiological and biochemical aspects of the toxic effects of different concentrations of Cr (0.5-0.25g/kg soil) in snake gourd plants.

5. Conclusions

The toxicity induced by heavy metal chromium in snake gourd seedlings were measured by analysing various morphological and physiological parameters The plant morphological characteristics such as shoot length, root length, number of leaves, leaf area, fresh weight, dry weight and tolerance index were decreased by Cr treatments in snake gourd seedlings. Likewise, the toxicity induced by Cr resulted in significant decrease in photosynthetic pigments chlorophyll and carotenoid contents.

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7. References

1. Ali MM, Hossain D, Al-Imran M, Khan S, Begum M, Osman MH. Environmental Pollution with Heavy Metals: A Public Health Concern. InHeavy Metals-Their Environmental Impacts and Mitigation 2021. IntechOpen.