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# Sodium Chromate Influence on Seed Germination and Seedling Growth of Horse Gram (*Dolichos Biflorus Linn.*)

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## ABSTRACT

Medicinal plant *Dolichos biflorus* L. used in traditional medicine is well known for its healing properties. Its seeds are majorly used in treatment of various ailments besides used as tonic, astringent, and diuretic. The main purpose of this study is Sodium Chromate (Na2Cro4) contamination in water, soil and plants is a serious health problem throughout the world. We studied the effect of aqueous solutions of 0, 0.5, 1.0, 1.5, 2.0, and 2.5 and 3.0 % Sodium Chromate on seed germination and seedling growth of *D. biflorus* at the end of 20days of treatment, significant reduction in root length, shoot length, seedling length, Rooting length/ shoot length ratio (cm) and fresh weight, % of germination were observed at increasing Chromate concentration. Response of *D.biflorus* varied on all growth parameters viz root, shoot, seedling length, dry weight and root / shoot ratio. There was also reducing rate of seed germination of *D. biflorus* with all treatment of chromium was recorded. The treatment of chromium at 3% produced significant 2.4 cm length of plant with effects on root, shoot and seedling length of *D. biflorus* as compared to control. The reduction in the seedling dry weight of *D. biflorus* at 3% of Sodium chromate was reduced and was more prominent with the increase in concentration at 3% of Sodium Chromate treatments. Tolerance indices and seedling vigor index of 3% for chromium treatment decreased with the increase in chromium concentration in the substrate as compared to control. More reduction in seedling tolerance and seedling vigor indices percentage of *D. biflorus* was recorded at 1.0 % for chromium treatment. There was further reduction in seedling vigor and tolerance indices of *D. biflorus* at 3% of Sodium chromate concentration as compared to control.

**Key Words:** Sodium Chromate and Seedling growth, Root length, Shoot length and Leaf length

## INTRODUCTION

*Dolichos biflorus* Linn (Fabaceae), is commonly known as Muthira in Telugu and horse gram in English. It is a branched, and dowsing herb, sub-erect or trailing annaul, with small trifoliate leaves, bearing, when mature, narrow, flat, curved pods, 1½ - 2 inches long, tipped with a persistent style. The pods contain 5-6 flattened, ellipsoid seeds, 1/8 - 1/4 in long. The plant is native of India and is distributed throughout the tropical regions of the old world. It occurs all over India up to an altitude of 5000 feet. It is an important pulse crop particularly in Madras, Mysore, Bombay and Hyderabad.

Chromium is present in food and feed plants, but the form is not well characterized (Cary, 1982; Das *et al*., 2005). The likely form is soluble in chromium (III) organic compounds such as chromium (III) oxalate in plants (Smith *et al*., 1989). Chromium is an important micronutrient for animals and humans (Bahijri and Mufti, 2002). Humans must consume organically bound or chelated chromium as part of the proper metabolism of Glucose Tolerance Factor (GTF). Although chromium (VI) can be rapidly absorbed through the intestinal wall, any ingested chromium (VI) is believed to be quickly reduced in the stomach where the pH is around 1 and numerous organic reducing agents can be found.

Chromium plays a key role in the biological life but above critical level it is toxic (Balamurugan *et, al*., 2004; Han *e, al*., 2004;) mutagenic (Gili *et,al*., 2002; Puzon *et, al*., 2002; Wise *et, al*., 2005), carcinogenic (Codd *et, al*., 2003; Reddy *et, al*., 2003; Sato *et, al*., 2003) and teratogenic (Asmatullah *et, al*., 1998). Trivalent form of chromium is more common and its compounds are less soluble

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and less toxic than hexavalent chromium (Smith. E and K. Ghiassi, 2006). Trivalent chromium forms stable complex with legends on DNA, proteins and small molecules such as glutathione (Adach and Cielak-Golonka, 2005). Trivalent chromium bounds to the DNA template cause increased DNA polymerase processivity and decreased DNA replication fidelity. These alterations in DNA function can result in greatly increased bypass of oxidative DNA lesions, which are promutagenic (Adach and Cielak-Golonka, 2005).

*D. biflorus* belongs to the family fabaceace. It was formerly called as Horse gram its vernacular names are Papataku in Telugu, Badachandrika in Hindi and Pampukaalchchedi in Tamil. (Mathews, 1983) have reported the high Chromate concentration in ground water at Hyderabad Telangana state, The aim of the present study was to evaluate the effects of varying concentration of chromium on seed germination and seedling growth performance of legume crop *D. biflorus*. The results of the study could be useful as selection criteria for cultivation in chromium-contaminated areas.

## MATERIAL METHODS

The healthy legume seeds of horse gram *D. biflorus* were collected from the Agriculture Research Station Hyderabad) at Botanical garden, Osmania University Campus Hyderabad and soaked in distilled water. The percentage of germination was first checked. To prevent fungal contamination, seeds of the *D.biflorus* were surface sterilized using dilute Sodium hypochlorite for one minute. The seeds were washed with double distilled water and placed in Petri plates on filter paper (Whatman No.42) at room temperature. Twenty seeds were placed in each Petri plate for replicates. Solutions of chromium salt as Sodium chromate were prepared having five 0, 0.5, 1.0, 1.5, 2.0 and 3.0 percentage of concentrations for treatment. The concentration of zero (0) served as control. The experiment starts with 5 ml of metal solution of 0.5, 1.0, 1.5, 2.0, and 3.0 concentrations to each set of respective treatment was applied. After every two days, 2ml of 0.5, 1.0, 1.5, 2.0, and 3.0 percentage solutions of chromium were added to respective treatment. The control received only 2ml of distilled water on alternate days. The experiment was designed on the basis of three replicates, the Petri dishes were kept at room temperature (32±2°C) with 240 Lux light intensity, and the experiment lasted for 10 days. The experiment was completely randomized. Seed germination, root, shoot, seedling lengths and root/shoot, ratios were recorded. The seedling dry weight was determined by drying the 3 tallest seedling from each replicate for each concentration, the one having good growth and placing the seedling in an oven at 80°C for 24 hours. Seedling dry biomass was measured with electrical balance.

Analysis of Variance (ANOVA), standard error and Duncan's Multiple Range Test (DMRT) to determine the level of significance at $p < 0.05$ on personnel computer using COSTAT version 3, statistically analyzed the seed germination and seedling growth data.

Tolerance indices of seedlings were determined with the help of the following formula.

$$\text{Tolerance indices (T.I.)} \ \frac{\text{Mean root length of treated seedlings}}{\text{Mean root length of control seedlings}} \times 100$$

## RESULTS AND DISCUSSION

Sodium Chromate treatment also produce significant effects on seed germination percentage of *D. biflorus* as compared to control (Table -1). Chromium treatments at 0.5 % significantly ($p<0.05$) affected root, shoot and seedling growth of *D. biflorus* as compared to control. The results indicated that root was strongly affected by all concentration of chromium treatments as compared to shoot length of *D. biflorus*. The results for shoot length of *D. biflorus* showed similar trend as in case of root growth. With the increase in concentration of chromium at 3% profound effects on seedling length of *D. biflorus* were recorded. Seedling size of *D. biflorus* which includes the length of root and shoot was recorded as compared to shoot length of *D. biflorus*. The results for shoot length of *D. biflorus* showed similar trend as in case of root growth. With the increase in concentration of chromium at 3% a profound effects on seedling length of *D. biflorus* were recorded. Seedling size of *D. biflorus* which includes the length of root and shoot was recorded as 6.5 cm for control and which decreased to 5.5 cm, 4.0 cm, 3.8 cm 3.0,2.8 and 2.4 cm when treated with 0.5, 1.0,2.0 and 2.5 % of Sodium chromate solution, respectively (Plate-I). A gradual decrease in seedling dry weight of *D. biflorus*, was recorded when treated with different concentration of chromium as compared to control. The seedling dry weight of *D. biflorus* was significantly decreased with increase in concentration up to 3.0 % of Sodium chromate.

The seedlings of *D. biflorus* showed different percentage of tolerance to Sodium chromate treatment as compared to control (Fig. 1). A high percentage of tolerance to chromium treatment at 0.5 % for *D. biflorus* as compared to control was recorded. The better percentage of chromium tolerance indices for *D. biflorus* seedlings was recorded at 1.0%. The lowest percentage of seedling germination indices for *D. biflorus* was recorded at 3% for Sodium chromate treatment. The Seed germination in seedlings of *D. biflorus* to chromium treatment were reduced with the values 95, 93, 90, 85, 84 and 82 percentage of seed germination when treated with 0.5, 1.0,2.0, 2.5 and 3% Sodium chromate concentration as compared to control, respectively.

**Table I: Effects of different concentrations of Sodium Chromate on different growth parameters of D. biflorus**

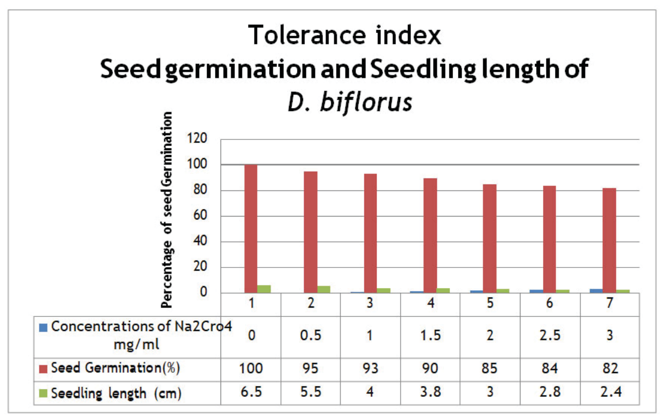
| Concentrations of Na2Cro4 mg/ml | Seed Germination (%) | Shoot length (cm) | Root length (cm) | Seedling length (cm) | Seedling dry weight (gm) | Rooting length/ shoot length ratio (cm) |
|---|---|---|---|---|---|---|
| Control | 100 | 2.5 | 4.0 | 6.5 | 0.9 | 1.6 |
| 0.5 | 95 | 2.0 | 3.5 | 5.5 | 0.7 | 1.75 |
| 1.0 | 93 | 1.8 | 2.8 | 4.0 | 0.6 | 1.5 |
| 1.5 | 90 | 1.2 | 2.6 | 3.8 | 0.63 | 2.1 |
| 2.0 | 85 | 1.0 | 2.0 | 3.0 | 0.5 | 2.0 |
| 2.5 | 84 | 0.9 | 1.9 | 2.8 | 0.45 | 2.1 |
| 3.0 | 82 | 0.8 | 1.6 | 2.4 | 0.4 | 2.0 |


**Plate I:** Effect of Sodium Chromate treated on seedling growth of *D.biflorus* effect of root, shoot and Seedling length on Control (A) treated 0.5(b), 1.0 (c), 2.0(d), 2.5(e) and 3%(f) Sodium chromate concentration.

## DISCUSSION

The increasing concentration of Sodium Chromate effect on morphological determining Chromate causes reduction in root length and shoot length due to unbalanced nutrient uptake by seedlings in presence of Chromate (Sabal *et, al.,*2006). Fresh weight and % of seedlings decreased monotonically with increasing Chromate concentration due to reduction of metabolic activity in presence of Chromate (Because germination is a one kind of metabolism and Chromate acts as a metabolic inhibitor (Gulzar and Khan, 2001; Gupta *et al.,* 2009; Sabal *et al.,* 2006).

This study provides evidence that the application of various concentration of chromium contributes to decreased seedling growth in Horse gram. There was less significant reduction in seed germination percentage of horse gram was observed which might be due to its resistance to chromium at all concentration to some extent. Germination and seedling establishment are critical stage in the life cycle of plant and can be affected in the presence of high level of metals in the immediate environment. Heavy metals have specific function and role in plant growth. Chromium is toxic heavy metal and easily available in air, water and soil. It was observed that among the heavy metal, chromium was found toxic at higher level. The plant under stress conditions are most likely to be adversely affected by heavy metals treatments. In the present studies, the toxicity of chromium at 0.5, 1.0,2.0, 2.5 and 3% on seedling growth and yield performances of *D. biflorus* were significantly affected. *D. biflorus* was subjected to different concentrations of chromium. The root growth of *D. biflorus* was more affected with the Cr treatment as compared to shoot. A significant inhibition in root length of *D. biflorus* was found at 3% as compared to control.




Excessive foliar spray of sodium Chromate reduced growth and development of litchi plants (*Litchi chinensis*) and also inhibited pollen germination (Zhang *et al.,* 1998). Growth and yield of wheat (*Triticum aestivum*) was severely affected by sodium Chromate (Singh *et,al.,* 2001). Sodium Chromate decreased the fresh and dry weights of the growing embryo axis of maize seedlings (Nagoor, 1997).

In another study, the toxic effect of $PbCl_2$ at 3% on the root growth of lentil (*Lens culinaris*) was recorded (Kiran *et.al.,* 2005). The roots of *P. oleracea* seedlings were more sensitive to heavy metal in comparison with shoot (Naz *et.al.,* 2013) . The results for shoot length showed similar trend as in case of root growth, the shoot length of *D. biflorus* decreased with the reduction in root length, which might be due to decreased in nutrients and water uptake from the substrate. The seedling size, which includes the length of root and shoot, was greatly decreased when treated with 3% of chromium as compared to control. Tolerance in seedlings of *D. biflorus* was decreased with the increase of chromium.

The results also showed that seedling dry weights of *D. biflorus* were also declined with increased concentration of chromium treatment. Essential and non-essential heavy metals generally produce common toxic effects on the production of low biomass, photosynthesis, alteration in water balance and nutrient assimilation (Singh *et.al.,* 2015). The present investigation confirmed that seed germination and seedling growth was affected under different concentrations of chromium.

The response of horse gram seedlings at optimum dose of chromium at 3% can help in understanding the tolerance limit to chromium stress. In addition to growth inhibition of *D. biflorus*, chromium treatment reduced biomass production. The effects of Cr has been reported in several studies over the last few years. At the cellular level, oxidative stress have been reported as a common mechanism in both stress situations (Smeets *et.al.,* 2009). All results treated with different concentration of chromium when compared with the control treatment showed reduction in seedling and vigor indices of *D. biflorus* and agreed with the findings of other researchers. Chromium treatment at 100 mg kg-1 in pot adversely affected seedling growth, and seedling vigor index and biochemical attributes of *Hibiscus esculentum* L. (Amin *et.al.,* 2013). It was found that lemon grass (*Cymbopogan flexuosus*.) did not tolerate Cr beyond 50 ppm in pot culture experiment (Patra *et al.,* 2014).

## CONCLUSION

At different concentration of chromium toxic effect on seedling growth of *D. biflorus* was observed and significant reduction in yield parameters as compared with control treatment. The tolerance and seedling vigor index of chromium treatment decreased with increase in chromium application. The difference in tolerance to chromium toxicity should be considered while cultivated in chromium contaminated areas and to cover the risk of chromium reference role in food chain. It was also concluded that chromium concentrations at 1.0 and 3% have negative effects on germination and seedling growth of horse gram. High Cr concentration (3%) caused more damage.

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