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STUDIES ON PHYSIOLOGICAL AND METABOLIC RESPONSES IN HORSE GRAM (*DOLICHOS BIFLORUS* L.) INDUCED BY CHROMIUM

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ABSTRACT

The current investigation looks on the phyto-toxicity and accumulation of chromium (VI) in Horse gram (*Dolichos biflorus* L.). Horse gram plants were subjected to chromium (VI) concentrations of (0.00, 0.05, 0.10, 0.20, 0.25, and 0.30 mg/L) at various concentration levels. At higher chromium (0.30 mg/L) concentrations, the foliar toxic symptoms of older leaves included loss of turgor and chlorosis, which eventually manifested as chlorosis in middle-aged leaves after Crizotinib therapy for five days (VI). Chlorosis signs became more significant and twisted to cytotoxicity in places with tapering membrane, slender strands, or weak folding qualities later in the stage, which were characterized by tapering lamellae, thin lenticels, and weak knotting property. On horse bean, Cr (VI) toxic effects has been discovered as reduced crop production, photosynthetic capacity, physiological as well as molecular action, and metabolic dysfunction are all negatively impacted. Iron uptake and accumulation in leaves were reduced by Cr (VI) (0.30 mL/L) in comparison to the control (From 15.60 to 4.35 grammes per kilogramme of dry weight), while boosting sulphur and phosphorous uptake and deposition. When the plants were subjected to the pretreatment for 29 days, the leaves accumulated the greatest amount of Cr (92.12 grammes per kilogramme of dry weight to 10.08 grammes per kilogramme of dry weight), followed by the roots (96.50 grammes per kilogramme of dry weight to 30.20 grammes per kilogramme of dry weight). When consumed in large quantities, the toxic Cr buildup and nutritional deficits that result from the intake of Cr-containing horse gramme can be hazardous to population health, according to the World Health.

Keywords: Chromium, Bioaccumulation, Phytotoxicity, Ribonuclease, Iron, Translocation Physiological and metabolic responses, Horse gram.

1. INTRODUCTION

Because of the vast number of uses for which it is used, chromium is widely regarded as a dangerous metal by the general public. It is the sixth more common metal in the earth's crust, after alumina and calcite [1]. Leather tanning and finishing [2], resistant iron manufacturing, coloring agents, digging muds, metal plating cleaning agents [2], catalysts and their components, catalytic oxidation synthesis, and specialized chemicals are just some of the industrial applications for chromium. Significant amounts of readily available Chromium (III) or Chromium (VI) have been found in the soil [3]. There are two types of chromium: elemental form chromium and tetravalent chromium (1), both with oxidation values ranging from -2 to +6, are the most frequent environmental forms of chromium. Chromium in its hexavalent chromate form [Chrome (VI)] is by far the most prevalent form found in nature [4]. In terms of movement, solubility, and cytotoxicity, chromium (III) and (VI) are two different types of chromium.

Heavy metal (HM) pollution of the environment appears to have become a common concern, and it appears to be related to anthropogenic activities like as mine extraction, melting, plating, in the fields of health as well as hydrocarbons silt removal as well as processing processes are also included in this category. It presents itself in a multitude of ways: the atmosphere that breathes, the liquid that consume, the land on which we produce All of our everyday habits, from what we eat to how much noise we hear, have an effect on our health

and shorten the lives of people who inhabit them [5]. Wastewater from factories is a major source of heavy metal contamination - with some facilities releasing harmful heavy metals into the surrounding environment consequently. A combination of alkaline, ammonium, chlorophenols, the heavy metals (HMs) are present in the effluents emitted by paper mills and fertilizer companies, and these pollutants contaminate water supplies and groundwater. Heterocyclic amines (HMs) can be found in substantial numbers in the waste water from a variety of industries including the dyes and pigments industry as well as the films and photographic industry as well as primarily based, metals washing, metalworking, leatherette, even extraction [6].

According to government estimates, India's dyeing industry annually releases around 2000-3200 tonnes of chromium into the atmosphere [7]. The control of leather tannery effluent discharge has become an unsettling problem, despite the fact that the chromium concentration of the effluent in Asian economies such as India normally ranges between 2000 and 5000 mg/mL, based on the Global Health Foundation's findings. As a result, the poisoning of water resources by chromium-containing effluent poses a major hazard to human and animal health and welfare [8]. Crops polluted by chromium accumulate, and this poses a health risk to humans through the consumption of Food tainted with chromium [9, 10]. The procedures by what types of plants are there obtain chromite are unknown because it is not required for plant development or processing. The oxidation states of Cr, as well as its bioavailability, absorption, translocation, and bioaccumulation, all influence its phytotoxicity. Plants, like animals, are capable of translocating and accumulating Cr in various amounts. Tiwari and colleagues [11] were among the several researchers who contributed to this study, which included: Several studies conducted [8, 10, 12] examined the effects of Cr on phytotoxicity in a variety of agricultural crop plants. Crop advancement was retarded, seed germination was inhibited, and biological changes were observed. Chromium's phytotoxicity also resulted in decreased pigment synthesis, important nutrients induce oxidative stress due to their transport, associated with a high, oxidative stress, and antioxidant enzyme activity [11, 13, 14]. Additionally, as previously mentioned, Cr is capable of altering the ultrastructure of a photosynthetic membrane [15]. Active mechanisms in plants transport Crustaceans (Cr) using anions like sulphate as transporters [6]. Chromium has been shown to change the uptake and accumulation mechanisms of critical in the plasma membranes of cells and animals nutritionally substances including such ammonia phosphate potash ferrous metal nickel cobalt aluminium copper.

The present study aimed to analyze the phytoconstituents expressions of the plant, metabolic and physiological features of crucial competitive concentration levels in horse bean (*Dolichos biflorus* L.), a widespread growing crop with nutritional content around the world. The research focuses on the generation of yields, antioxidant activities, nutrient absorption, transportation behavior, and concentration of nutrients under chromium stress created by Cr (VI).

2. MATERIAL AND METHODS

Method [16] which is suitable for the Indian climate patterns, used for the analysis of Horse gramme (*Dolichos biflorus* L.) They used an adaptation to approach [16] that was practicable for the Indian climate patterns [17] to grow in sand cannabis culture (processed) under greenhouses settings at slightly not just acidic (6.8 to 7.0), but also the heat is transferred [17]. For the purpose of cultivating the Horse gramme, the following nutrients were added to distilled water to create a well-balanced nutrient solution: 1 mM $CuSO_4$ and 1 mM $ZnSO_4$, 0.2 mm Sodium Molybdate, Molybdic Acid, 0.1 mM $NISO_4$, 4 mMCa (NO_2), 0.2 mm Na_2 $MO0_4$, 0.1 mM $NISO_4$, 2 mM $MgSO_4$, 1.33 mM NaH_2PO_4, 100 mM Fe-EDTA, 10 mM $MnSO_4$, 30 mM HBO_30, 0.1 mM COSO, and 0.1 mM NaCl were used in the reaction mixture. 4 mM KNO_2, to maintain nutritional balance as a chelate, iron was provided in the form of Fe ethylene diamine triacetic acid (Fe-EDTA) is a chemical compound that is used to treat ferric ethylene diamine triacetic acid [18]. In order to maintain uniform plant growth, it was required to re-pot the experimental pots with freshly manufactured nutrient solution on a consistent basis. Additionally, each pot was flushed weekly with distilled water in order to remove absorbed nutrients and potentially harmful material from the root zone system. As a result of the use of buffers, the pH of the nutrient solution was brought down to 6.8+0.2 in order to provide adequate nutrition to the plants during the experiment. For the duration of the experiment, distilled water was utilized to water the plants as and when they were required. Various doses of Cr were applied to Horse Gramme plants as potassium dichromate (AR grade salt) form and controls were treated with nutritional solution (after 40 days of development). The Cr values are 0.00, 0.05, 0.10, 0.20,

0.25, and 0.30 mg/L, with 0.00 being the lowest and 0.05 being the highest (without Cr). Throughout the study, plants were evaluated on a regular basis for cytotoxicity and development reactions and the results were consistently positive.

In this study, the relative water content (RWC) of Horse gramme central leaf was assessed on day 47 (7 days after Cr delivery) after the plant was planted [18]. Everything was done between the hours of 9 and 11 in the morning in a humid atmosphere of sand, using pots filled with nutritious solution. Each measurement was taken twice. Throughout the trial, the temperature and humidity in the glasshouse remained between 35 and 40°C and 65 and 75 percent, respectively. The leaf area (cm^2) of the treated plant was measured with the Delta-T leaf area measurement instrument 12 days just after chrome therapy in order toanalyse the growth behaviour of the treated plant. In Horse gram's primitive leaves isolate, the concentration level of chlorophyll (a), chlorophyll (b), and total chlorophyll, as well as the levels of sugar, carbohydrate, nitrogen, catechol, enzymatic activities (carbonic anhydrase, peroxidase, and ribonuclease) and soluble protein content were determined at 48 days (8 days following Cr treatment) following harvesting (Table1). The treated Horse grame plants were harvested after 69 days of growth in order to conduct a steel test of the plants histopathology, that they had obtained, was performed (29 d after Cr treatment). Specimens of the plants were picked and washed with 0.01 N HCI before ever being washed with deionized water for multiple times. Using tap water and eventually distilled water, the roots, shoots, and leaves were separated and used accordingly. A constant weight was achieved by chopping and drying separated plant pieces at 70°C for up to an hour in order to achieve a constant weight, after which they were weighed. Flowers that have been dried out were used in the Piper method, samples (100 mg) were digested with HCIO HNO_3 (1:4 v/v) and diluted with milli-Q water before being tested. During their experiment, the research conducted an Inductively Coupled Analyzer (ICP Optima 3300 RL) from the Perkin Elmer Corporation to measure the amounts of heavy metals and cr present in various parts of plants and organs. Everything was done in triplicate, and all data was statistically evaluated to guarantee that there was no variance in the results or that they were legitimate in some kind. By using calorimetry, we were able to measure the amount of phosphorus in the water, and by using turbid metrication, we were able to determine the quantity of sculpture in the water (Table 1). According to scientists [19], the mean values are displayed along with the standard error of the mean.

2.1. High-quality ensure with manage the organization

The instrumental techniques were standardized and performed at Osmania University Hyderabad, Telangana State, India, provided reference standards supplies for iron (BND 1101.02 provided by the Physics Departmental Research lab, Osmania University Hyderabad, Telangana State, India) and Cr (BND 1101.02 provided by the Physics Departmental Laboratory, Osmania University Hyderabad, Telangana State, India) and other elements, Telangana State India) Germany's E-Merck provided samples from the National Environmental Company's quality assurance program. A frequent evaluation (n=6) of standard reference samples was used to normalize the Metal compounds' analytical data integrity is based on a variety of factors, with the results determined to be within 2.01 percent of the certified values for all of the samples evaluated. When it came to iron and chromium, the average resurrection rate was around 96% and 98% respectively. For each group of data, the blanks were run three times in order to ensure that the technique was proper for that particular subset of data. Both iron and chromium had detection thresholds of 0.3 and 0.5 parts per billion, respectively, according to the findings.

3. RESULTS AND DISCUSSION

In order to determine whether or not high concentrations of Cr (0.00, 0.05, 0.10, 0.20, 0.25, and 0.30 mg/L) caused toxicity in Horse gramme plants, a set of control pots were used in conjunction with a set of experimental pots to determine whether or not potash dichromate analytical grade sodium, which was provided as potassium dichromate AR grade salt, caused toxicity in Horse gramme plants.

The results showed that the plants were not harmed by the excess levels of Cr supply. Horse gramme plants were grown and kept to maturity, and the toxic effects of varying levels of chromium (VI) stress were observed in terms of observable phytotoxic symptoms and Horse gramme. The behaviour of growing plants, as well as the growth of other species, are all studied. On the fifth day of Cr supply, chlorosis was discovered on old leaves as a result of an excess of Cr (0.50 mg/L) in the solution, which was caused by an excess of Cr (0.50 mg/L). Toxic effects were observed as wilting of leaves that eventually hung from the petiole after the seventh day of treatment

with increasing dosages of Cr (0.20, 0.25, and 0.30 mg/L), which was observed after the seventh day of treatment.

Mostly on tenth day of Cr provision, the colour of the old leaves treated plants changed to a brilliant yellow. In the following few days, foliage in terms of percentage, height, and shape decreased, the plant succumbed to necrosis as the lesion progressed. Large necrotic areas developed in the damaged leaves as a result of the accumulation of necrotic patches. Chlorotic leaves become wilted and dried over the course of a few days, eventually resulting in premature leaf fall. During the experiment, similar symptoms were noticed in both the middle and upper young leaves. Plants grown at a lower concentration of Crafter on the 14th day of treatment showed a significant delay in the onset and growth of chlorosis in the leaves compared to the higher percentage plants.

3.1. The effects of Chromium on the weight, grain yield, number of leaves, and water uptake of Horse gramme

As depicted in Graph1, overall impact of Cr treatment on the Horse Gramme plant's biomass, grain output, number of leaves, and total water uptake as well as its total relative water content are all shown. The dry biomass of Horse gramme reduced progressively as the quantity of Cromium (VI) following the results of the experiment, the amount of thiamine in the nutritional solution increased between 0.05 to 0.25 mM delivery. After 69 days of treatment with excessive Cr (0.25 mg/L), the biomass of the treated plant decreased by 73.07 percent in comparison to the control plant. For productivity purposes, only yields of the concentrations of significance level and 0.10 mg/L Cr (VI) was attained; greater concentration levels (0.25 mg/L) did not result in the formation of pods. When straw yield decreased significantly as compared to the controls crops when exposed to Cr concentrations of (0.05 and 0.25 mg/L), with a bigger decline observed when the Cr concentrations are greater (0.25 mg/L). It was discovered that the dimensions of the seeds were abnormal, with seeds that were twisted and shrivelled in plants that had been exposed to higher levels of Chromium. Horse gramme was treated with 0.25 mg/L Cr (VI) at a concentration of 0.25 mg/L, resulting in a visible grain yield weight loss of 76.95 percent, according to the current experiment. At day 52ndwhen compared to the control plant (12 days after Chromium therapy), the leaf area of Horse gramme plants reduced as the amount of Cr applied increased. At a concentration of 0.25 mg/L of Cr treatment, the depression in leaves was found to be 56.16 percent less when compared with control group (Fig.1). When comparing Chromium medicated foliage to control plants with an increase in corrosion rate, the moisture content of the untreated leaves declined significantly more quickly.

Table 1: Measurement variables and reference procedures that have been modified

S. No	Dimension	Methodology as a point of comparison
01	Phosphorous	[20]
02	Sulphur	[21]
03	% comparative groundwater	[18]
04	Chloroplast tensile	[22]
05	Hillwork	[23]
06	Protein	[24]
07	Glucose	[25]
08	Ammonia	[26]
09	Carbohydrates	[27]
10	Coagulated	[28]
11	Superoxide dismutase	[29]
12	Ribo nuclease	[30]

Table 2: The potentially toxic consequences of varied chrome treatment on the energy, grain production, number of leaves, and water uptake of horse gramme plants

Hours of develop-ment	some few days later	Mg/L Cr							LSD (P-0.05)
69	29	**Biomass:g**	**control**	**0.05**	**0.10**	**0.15**	**0.20**	**0.25**	**0.30**	
		Plant	15.60	10.90	6.70	5.25	6.25	4.20	4.35	0.78
69	29	Grains : g plant -1	4.86	2.15	1.12	0.82	0.72	0.62	0.42	0.23
52	12	Leaf area : cm^2	92.12	75.10	57.59	40.38	30.38	20.38	10.08	2.56
47	07	RWC: %	96.50	90.50	56.30	39.20	36.20	32.20	30.20	3.51

The results are the means plus standard error of the average (n-5)

As compared to the control, excess Cr (VI) (0.30 mg/L) reduced iron uptake and development in the leaves (from 15.60 to 4.35 g-1dw), while simultaneously increasing sulphur and phosphorus uptake and accumulation. After 29 days of treatment, the leaves accumulated the greatest amount of Cr (92.12g g-1dw to 10.08g g-1dw), followed by the roots (96.50g g-1dw to 30.20g g-1dw) and the stems (92.12g g-1dw to 10.08g g-1dw). When consumed in large quantities, the toxic Cr buildup and nutritional deficits that result from the intake of Cr-containing horse gramme can be hazardous to population health, according to the World Health Organization.

It was important to formulate plants of the horse gramme species to perfection while providing them with critical nutrients in order to compare grain crop productivity under chromium stress to that produced under control conditions. The deficiency symptoms inside the upper part of the leaf on aged foliage part of the leaves were identified as an obvious biologic indication of excessive Cr (0.25 mg/L) in the environment after the fifth day of Cr supply, as observed in the laboratory (at day 45). The visual signs emerged as wilting of afflicted leaves that then hung loose from the petiole after 7 days of Cr administration at the concentrations the concentrations of 0.1 and 0.25 mg/L for Chromium, respectively. This plant's previous leaves changed from a dull green to a dazzling golden yellow on the 10th day of its metal supply. Within the same time period, there was a decline in the type and range of leaflets, the deficiency symptoms became more severe and eventually necrosis in the following days, spots of cytotoxicity aggregated, resulting in large rotting regions, and leaves appeared chronically wilting as drying, culminating in early leaf fall. In the literature, this is the first time that observable potentially toxic effects of high Chromium within Horse grame plant species have been recorded, and they appear to be comparable to those described in Citrullus plants [10, 31]. Researchers [32] looked at the growth of Cabbage flowers, as well as discovered that there is a development slowdown as a common sign of having too much Chromium in the environment. Biological effects of generated chromium radiation on energy, food, number of leaves, and other parameters. As the concentration of chromium in the necessary nutrient rose throughout the current experiment, the amount of plant biomass, grain yield output, number of leaves, the RWC (Table 2) decreased in Horse gramme plants. Increased shoot and root growth could be responsible for the decrease in Horse gramme plant biomass. As a result of this, only tiny amounts of critical minerals as well as moisture are transported to plant shoot portions through the plant roots. It was discovered by scientists [10, 33] that when the Cr concentration of the plant increases, the leaf area decreases.

In the form of seeds introduced to Cr, numerous experimental ideas have been developed to explain how the nutrient causes biochemical degradation with alterations in various physiological systems, such as decreased seedling establishment and development, leaf senescence, slowing, and eventually going extinct, are a fact that was noticed [34, 35]. According to other research, excess Cr had a severe effect on physiological and biochemical processes, and as a result, crop production and productivity were both adversely affected at the same the passing of time [10, 12]. The RWC in Horse gramme was reduced by 18.5 percent, 37.5 percent, and 56.2 percent when the concentration of Cr was 0.05, 0.1, and 0.30mg/L, respectively. Reduced fresh biomass in Horse gramme plants as a result of increased moisture loss, as demonstrated by low RWC in leaves, which resulted in the leaves' look becoming wilted. Those plant leaves that received an excessive amount of Cr (VI) had reduced water centennial and relative water content [11, 12].

4. CONCLUSION

With this study it is concluded that Horse gram (*Dolichos biflorus* L.) when subjected to chromium (VI) at different concentrations (0.00, 0.05, 0.10, 0.20, 0.25, and 0.30 mg/L), 0.30 mg/L shown foliar toxic symptoms of older leaves included loss of turgor and chlorosis, which eventually manifested as chlorosis in middle-aged leaves after Crizotinib therapy for five days (VI). Chlorosis signs became more significant and twisted to cytotoxicity in places with tapering membrane, slender strands, or weak folding qualities later in the stage, which were characterized by tapering lamellae, thin lenticels, and weak knotting property. On horse bean, Cr (VI) toxic effects has been discovered as reduced crop production, photosynthetic capacity, physiological as well as molecular action, and metabolic dysfunction are all negatively impacted. Iron uptake and accumulation in leaves were reduced by Cr (VI) (0.30 mL/L) in comparison to the control. (From 15.60 to 4.35 grammes per kilogramme of dry weight), while boosting sulphur and phosphorous uptake and deposition. When the plants were subjected to the pretreatment for 29 days, the leaves accumulated the

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Conflict of interest

The authors declare that they have no conflicts of interest.

6. REFERENCES

1. Wakeel A, Xu M, & Gan Y. *Int. J. Mol. Sci*, 2020; **21**(3):728.
2. Nriagu J O, Nieboer E. *Am. J. Plant Sci,* 1988; **17:**15
3. Chapman H D. *Plant Soil Environ,* 1966; **60-61**:150-179
4. Ashraf A, Bibi I, Niazi N K, Ok Y S, Murtaza G, et al. *Int. J. Phytoremediation,* 2017; **19(7):**605-613.
5. Datta J K, Bandhyopadhyay A, Banerjee A, Mondal N K. *J Agric Technol,* 2011; **7(2)**:395-402.
6. Cervantes C, Campos-García J, Devars S, Gutiérrez-Corona F, Loza-Tavera H, et al. *FEMS Microbiol. Rev,* 2001; **25(3)**:335-347.
7. Zayed A M, Terry N. *Plant Soil Environ,* 2003; **249(1)**:139-156.
8. Vajpayee P, Rai U N, Ali M B, Tripathi R D, Yadav V, et al. *Bull Environ Contam Toxicol,* 2001; **67(2)**:246.
9. Broadway A, Cave M R, Wragg J, Fordyce F M, Bewley R J, et al. *Sci. Total Environ,* 2010; **409(2):**267-277.
10. Tiwari K K, Dwivedi S, Singh N K, Rai U N, Tripathi R D. *J Environ Biol*, 2009; ***30*(3)**:389-394.
11. Tiwari K K, Singh N K, Rai U N. *Bull Environ Contam Toxicol,* 2013; ***91*(3):**339-344.
12. Dube B K, Tewari K, Chatterjee J, Chatterjee C. *Environ. Chem. Lett,* 2003; **53(9)**:1147-1153.
13. Poschenrieder C H, Vazquez M D, Bonet A, Barcelo J. *J. Plant Nutr.*, 1991; **14(4):**415-428.
14. Panda S K, Chaudhury I, Khan M H. *Biol. Plant,* 2003; **46(2)**:289-294.
15. Bassi M, Corradi M G, Realini M. *Cytobiosis*, 1990; ***62*(248):**27-38.
16. Hewitt E J. *Tech. Commun. East Malling, Commonw. Bur. Hort. Plant. Crops* 1952; **22:**241 202-32 pp.
17. Agarwala S C, Chatterjee C. *Adv Nutr; ADV NUTR.* 1996; **401-453:**
18. Barrs H D, Weatherley P E. Aust. J. Biol. Sci., 1962; **15(3)**:413-428.
19. Panse V G, Sukhatme P V. *Statistical methods for agricultural workers,* 1954; 361 pp.
20. Wallace T. *A colour atlas and guide. 2nd HMSO,* 1951;
21. Chesmin L, Yien C H. *Soil Sci. Soc. Am. Proc,* 1951; **15**:pp. 149-151.
22. Arnon D I. *Plant Physiol*, 1949; **24(1)**:1.
23. Brewer J M, Jagendorf A T. *Plant Physiol*, 1965; **40(2)**:303.
24. Bradford M M. *Anal. Biochem*, 1976; **72(1-2):**248-254.
25. Arndt R G. *J. Herpetol,* 1975; **9(4)**:357-359.
26. Swain T, Hillis W E. *J. Sci. Food Agric*, 1959; **10(1):**63-68.
27. Nelson N. *J. biol. Chem*, 1944; **153(2):**375-380.
28. Bisht S S, Sharma A, Chaturvedi K. Indian J Agric Biochem, 1989; ***2*:**109-115.
29. Luck H. *Catalase Academic press.* 1965; pp. 885-894.
30. Tuve T W, Anfinsen C B. *J. Biol. Chem,* 1960; **235(12):**3437-3441.
31. Tiwari K K, Dube B K, Chatterjee C, Sinha P. *Indian J Hortic,* 2008; **65(2)**:171-175.
32. Chatterjee J, Chatterjee C. *Environ. Pollut,* 2000; **109(1)**:69-74.
33. Pandey V, Dixit V, Shyam R. *Protoplasma*, 2009; **236(1)**:85-95.
34. Rai V, Vajpayee P, Singh S N, Mehrotra S. *Plant Sci,* 2004; **167(5)**:1159-1169.
35. Shanker A K, Cervantes C, Loza-Tavera H, Avudainayagam S. *Environ. Int*, 2005; **31(5)**:739-753.
36. Piper C S. *Soil Sci. Plant Anal,* 2019.
37. Jacobson L. Plant Physiol, 1951; **26(2)**:411.