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STUDIES ON EFFECTS OF SODIUM CHROMATE ON SEED GERMINATION AND SEEDLING GROWTH OF BLACK GRAM (*VIGNA MUNGO* L.) HEPPER

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ABSTRACTS:

Vigna mungo (L.) Hepper is a warm-season legume that is nutrient dense and contributes to food security. The availability of metals in the atmosphere due to industrial and human activities is a global problem. The main purpose of this study is to determine the sodium chromate (Na_2Cro_4) contamination in water, soil and plants is a serious health problem worldwide. At the end of 20 days of treatment 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 seed growth of Vigna mungo, significant reduction in root length, shoot length, seed length, roots Growing length / shoot length ratio (cm) and fresh weight, germination% were observed when increasing chromate concentration. Shoot, root, seed length, seed dry weight and *V. mungo* root / shoot ratio responded differently to all growth parameters. *V. mungo* with all treatments of chromium. The seed germination rate of mungo was also reduced. Chromium treatment at 3% produced a plant 2.4 cm tall with effects on root, shoot and seed length of *V. mungo* compared to control. *V. mungo* at 3% of sodium chromate. Dry weight loss of mung bean seeds was reduced and became more prominent with increasing concentration at 3% in sodium chromate treatments. Increased ration of chromium on the surface compared to control reduced tolerance indices and seed strength index of 3% for chromium treatment. 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 *V. mungo* was recorded at 1.0 % for chromium treatment. There was further reduction in seedling vigor and tolerance indices of *V. mungo* 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:

Urdbean *V. mungo* (L.) Hepper, black gram or black lentil) are often called “minor” crop, due to their restricted global social and economic significance, determined by their place in the world production, their underutilized use, and limited breeding efforts. The term also reflects their relatively narrow regional importance, which has not yet reached a global scale within the range of soil and climatic conditions suitable for them (Chiveng *et al.* 2015). Meanwhile these crops deserve close attention due to their high nutritional and fodder value, as new sources of ingredients for industry and pharmacology, and their utility in crop rotations. These “underutilized” crops, along with others, are under the constant attention of FAO—the Food and Agriculture Organization with a view to their promotion and large-scale contribution to agricultural production (Williams and Haq 2002).

V. radiata and *V. mungo* are closely related, formerly attributed to the genus Phaseolus and classified as one species. Both species have the same chromosome number (2n = 2x = 22) and similar karyotypes. There are partially fertile progenies in crosses when *V. radiata* is used as the maternal plant, but the reciprocal crosses have not been found viable (Sen and Ghosh 1963, Chowdhury *et al.*, 1977; Singh 1981). Together with dissimilarities in some morphological features (Burlyaeva *et al.,* 2016), cytological investigations revealed differences of the species in chromosome translocations, deletions and duplications (De Deepesh and Krishnan 1966). *V. radiata* and *V. mungo* have also been placed in separate botanical species by molecular markers (Jaaska and Jaaska 1989; Santala *et al.,* 2006).

Seed germination is usually the most critical stage in seedling establishment determining successful crop production (Shahi, *et.al* 2015a Bhattacharjee, 2008). The germination of seed is a complex process depending on the genetic and environmental factors; such as temperature, light and salinity (Mahmoud, 1985). Salinity adversely affects the plant growth and development, hindering seed germination (Shahi *et.al.,* 2015a). There are two basic ways in which salinity affects the plants. First high salt concentration in soil made it harder for plant roots to extract water from the soil. This is purely the result of osmosis, the movement of water across a semi-permeable membrane as in a plant cell, from an area of high-water potential (low salt concentration) to an area of low water potential (high salt concentration). When the concentration of soil-water salt stress above threshold water will tends to flow out of the plant. If plants had no way of regulating this process they would quickly dehydrate and die. Secondly in a saline environment salt enters the plant and accumulates and can reach toxic concentrations (Arnold, 2011).

Vigna mungo Closely related to *Vigna radiata*. There has been confusion on the taxonomic status of *Vigna mungo* and Vigna radiata; because they are closely related it was proposed that they be grouped into a single species. However, at present they are considered as 2 separate species with as major differences: flower color (bright yellow in *Vigna mungo*, pale yellow in *Vigna radiata*), pocket on the keel (longer in *Vigna mungo* than in *Vigna radiata*), fruit shape (pods of *Vigna mungo* are shorter and erect on the peduncle, in *Vigna radiata* the pods are longer and spreading or pendulous.

Black gram is an erect, hairy, bushy, annual plant with a well-developed taproot, growing 30 - 100cm tall. The stem is diffusely branched from the base. Sometimes the plant adopts a twining habit. The plant is often cultivated in many areas of the tropics, especially Asia, for its edible seed. It is also sometimes used medicinally and as a source of soap, as well as being grown as a green manure crop

A plant of the drier tropics, where it is found at elevations up to 2,000 meters. It grows best in areas where annual daytime temperatures are within the range 22 - 35°c, but can tolerate 8 - 40°c. The plant does not tolerate frost. It prefers a mean annual rainfall in the range 650 - 900mm, but tolerates 530 - 2,430mm. Rain at flowering time has a very adverse effect upon seed yields. Plants are not adapted to wet, humid areas with high rainfall, but can, however, be grown in the dry season of wetter areas so long as this is at least 4 months in duration.

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 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 and less toxic than hexavalent chromium (Smith and Ghiassi, 2006). Trivalent chromium forms stable complex with legends on DNA, proteins and small molecules such as glutathionein (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).

In view of the above, in the present study, effects of Sodium Chromate (Na_2Cro_4) stress on the seed germination and seedling growth of *Vigna mungo* was carried out with the following objectives. To identify the physiological and morphological responses of selected leguminous crop and to analyse the sensitivity and stress tolerance in selected crops.

MATERIAL METHODS:

The healthy legume seeds of mung bean *V. mungo* were collected from the market. The percentage of germination was first checked. The seeds were surface sterilized with dilute solution of Sodium hypochlorite for one minute to prevent any type of fungal contamination. The seeds were washed with double distilled water and placed in Petri dishes (90 mm diameter) on filter paper (Whatman No. 42) at room temperature. Twenty seeds were placed in each petri plate and there were three 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 start of experiment, 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, 2 ml of 0.5, 1.0, 1.5,2.0, 2.5and 3.0 percentage solutions of chromium were added to respective treatment. The control received only 2ml of distilled water on alternate days. The experiments were 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 seedlings 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.

Tolerance indices

$= Mean\ root\ length\ of\ treated\ seedlings = \times 100$

Mean root length of control seedlings

RESULTS AND DISCUSSION:

Sodium Chromate treatment also produce significant effects on seed germination percentage of *V. mungo* as compared to control (Table -1). Chromium treatments at 0.5 % significantly ($p<0.05$) affected root, shoot and seedling growth of *V. mungo* as compared to control. The results indicated that root was strongly affected by all concentration of chromium treatments as compared to shoot length of *V. mungo*. The results for shoot length of *V. mungo* showed similar trend as in case of root growth. With the increase in concentration of chromium at 3% profound effects on seedling length of *V. mungo* were recorded. Seedling size of *V. mungo* which includes the length of root and shoot was recorded as compared to shoot length of *V. mungo*. The results for shoot length of *V. mungo* showed similar trend as in case of root growth. With the increase in concentration of chromium at 3% a profound effect on seedling length of *V. mungo* were recorded. Seedling size of *V. mungo* 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 *V. mungo*, was recorded when treated with different concentration of chromium as compared to control. The seedling dry weight of *V. mungo* was significantly decreased with increase in concentration up to 3.0 % of Sodium chromate.

The seedlings of *V. mungo* 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 *V. mungo* as compared to control was recorded. The better percentage of chromium tolerance indices for *V. mungo* seedlings was recorded at 1.0%. The lowest percentage of seedling germination indices for *V. mungo* was recorded at 3% for Sodium chromate treatment. The Seed germination in seedlings of *V. mungo* 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.

DISCUSSION:

The increasing concentration of Sodium Chromate shows 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 *V. mungo* were significantly affected. *V. mungo* was subjected to different concentrations of chromium. The root growth of *V. mungo* was more affected with the Cr treatment as compared to shoot. A significant inhibition in root length of *V. mungo* was found at 3% as compared to control.

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 *V. mungo* 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 *V. mungo* was decreased with the increase of chromium.

The results also showed that seedling dry weights of *V. mungo* 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 *V. mungo*, chromium treatment reduced biomass production. The effects of Cr have been reported in several studies over the last few years. At the cellular level, oxidative stress has 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 *V. mungo* 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* Nees ex.steud. wats.) did not tolerate Cr beyond 50 ppm in pot culture experiment (Patra *et, al.,* 2014).

CONCLUSION:

It was concluded that the treatment of different concentration of chromium at higher concentration produced toxic effects on seedling growth of *V. mungo* along with significant reduction in yield production as compared to control treatment. Similarly, the tolerance and seedling vigor index to chromium treatment decreased with the increase in chromium application for *V. mungo* seedlings. 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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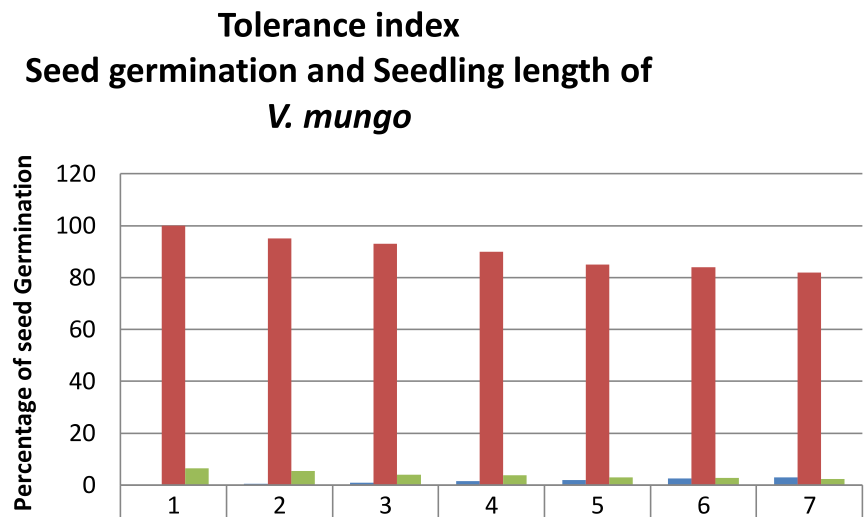
Table: I Effects of different concentrations of Sodium Chromate on different growth parameters of *V. mungo*

Concentrations of Na_2Cro_4 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 *V. mungo* 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.



	1	2	3	4	5	6	7
Concentrations of Na2Cro4 mg/ml	0	0.5	1	1.5	2	2.5	3
Seed Germination(%)	100	95	93	90	85	84	82
Seedling length (cm)	6.5	5.5	4	3.8	3	2.8	2.4

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).