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Research Article

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Assessment of Heavy Metals (Cd, Co, Cr and Pb) Effects on *In Vitro* Seed Germination and Seedling Growth of *Vigna mungo*

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Abstract: *Vigna mungo* generally called as black gram is a nutrient food crop which is widely used in Indian cuisine and also used in traditional ayurvedic medicine. By natural and anthropogenic activities, black gram growth is highly affecting by heavy metal pollution. Hence, it is necessary to conduct the experiments to know the effects of various heavy metals on growth of black gram in turn yield levels. In the current study, we aimed to study the *in vitro* effects of heavy metals such as chromium (Cr), cadmium (Cd), cobalt (Co) and lead (Pb) on germination of seeds and seedling morphology of black gram. The concentrations include 1, 10, 20, 50 and 100 ppm of respective heavy metal (Cr, Cd, Co and Pb) along with the two positive controls i.e. tap water (T.W) and distilled water (D.W) were used without any heavy metal for the preparation of media. Upon treatment, percentage of germination and both root and shoot lengths of black gram were significantly affected by increasing concentrations of cadmium, cobalt and lead when compared to controls. Specifically, germination percentage was reduced from 1 ppm to 100 ppm i.e. 77% to 46% with Cd, 64% to 50 % with Co and 78% to 70 % with Pb treatments. This data indicates that each heavy metal has its own impact on black gram seed germination. Similarly, shoot and root lengths were also reduced upon these heavy metal treatments. Although the percentage of germination decreased drastically (36% at 100 ppm) along with the increasing concentration of chromium, the root and shoot lengths were suddenly increased (hugely root length) when compared to controls. In conclusion, each heavy metal has its own critical point for each seedling or plant growth.

Keywords: Black gram, Cadmium, Cobalt, Lead, Chromium, Germination, Seedlings

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1. INTRODUCTION

Both biotic and abiotic stresses seriously influence the crop growth and yield. Heavy metal (HM) stress is one of the key abiotic stresses apart from drought, salinity, extreme temperature etc. The anthropogenic causes like urbanization, industrialization, usage of chemical fertilizers and pesticides, mining and quarrying activities are the main sources of heavy metals[1]. Generally they are classified into two groups based on the usage i.e. essential and non-essential. Soil was getting contaminated with both the types of heavy metals such as chromium (Cr), cadmium (Cd), cobalt (Co), lead (Pb), zinc (Zn), nickel (Ni),copper (Cu) etc. worldwide through both natural and anthropogenic reasons[1.] They are released continuously and the estimated amounts reached to metric tons for most of the heavy metals including Cr, Cd, Co and Pb in the past few decades[1-2]. Heavy metals can remain in the environment for longer periods and accumulated into plants, microorganisms and animals including human beings, gets biomagnified through food chain[3-4]. The high concentration of heavy metals in the soil will affect the crop plants adversely particularly seed germination and seedling growth by interfering with the activities of many key enzymes related to normal metabolic and developmental processes and alter the biomolecule levels of cells[5-6]. Also they affect the stomata function, photosynthetic activity and damage the root system[7-8]. In developing countries, the crops which are grown in the surroundings of heavy metal industries are affected with toxicity[9-10]. Cadmium is one of the more toxic and highly mobile element from soil to plants and then to food chain. The high concentration of cadmium can cause renal cancer and along with arsenic will lead to lung cancer[11]. Primarily, high amount of cadmium was observed in roots and a very low amount was transferred to shoots leading to the part damage of the root tissue. Specifically functions such as photosynthesis, nitrogen metabolism, water and nutrient uptake were reduced by the heavy metal cadmium[12]. In *Pisum sativum*, various oxidative stresses were observed in leaves and root tissues by different concentrations of cadmium[13]. In legume roots, during nitrogen fixation cobalt along with *Rhizobium* bacteria play a major role. But many studies reported that high levels of cobalt can inhibit pollen germination, pollen tube growth and germination which may lead to less growth of plumule and radicle[3, 14]. Lead is one of the severe toxic heavy metals and contaminated soils that can decrease the crop production[15]. Tomar et al[16] and Bekiaroglou and Karataglis[17] reported that high concentration of lead in the soil can reduce the shoot and root length, plant weight, root nodules and chlorophyll content in *Vigna radiata* and *Mentha spicata*. Chromium exists in two oxidative states like trivalent and hexavalent, but hexavalent is the main toxic pollutant source from different industries such as electroplating, leather tanning, textile preservation and metal finishing which damage the crop growth including yield[18]. With the enhancement of heavy metal formation, living organism specifically plants are frequently affected[2]. But the interruption in biochemical and molecular pathways of plants by these metals has not been clear for most of the heavy metals[7]. To achieve this, initial understanding of the mechanisms underlying the plant response to heavy metals is vital process. Hence in the current investigation, we aim to study the effects of chromium, cadmium, cobalt and lead individually on black gram seed germination and seedling growth under *in vitro* conditions. To the best of our knowledge, for the first time effects of four heavy metals on black gram was studied at a time to know the growth pattern.

2. MATERIALS AND METHODS

The local variety (LBG) of *Vigna mungo* seeds were collected for the treatment of different toxic heavy metals such as cadmium, chromium, lead and cobalt under *in vitro* conditions to examine their germination and seedling morphology and development.

2.1 *Preparation of media*

The media used for the current work were made by the heavy metal salts like potassium dichromate ($K_2Cr_2O_7$) for chromium metal, cadmium chloride monohydrate ($CdCl_2.H_2O$) for cadmium metal, lead nitrate ($Pb(NO_3)_2$) for lead metal and cobalt chloride hexahydrate ($CoCl_2.6H_2O$) for cobalt metal. By using above salts, stocks were prepared for 1000 ppm for further dilutions. From the stock solution, the required concentrations such as 1, 10, 20, 50 and 100 ppm were used for the preparation of different media as per Neelesh Babu et al[7]and Keerthi Kumari et al[8]. Along with test metals, tap water (T.W.) and distilled water (D.W.) media were used as two positive controls and pH was adjusted to 5.7 to 5.8. Finally 0.8% of agar was added to the media and was boiled to dissolve the agar followed by dispensed into the test tubes. The media was sterilized by autoclaving for 15 to 20 min at $15lb/in^2$ in an autoclave. After autoclaving, media tubes were kept in a slant position for more space to inoculate seeds.

2.2 *Preparation of seeds for inoculation*

The uniform seeds were selected for the experimental purpose and were washed under tap water for 4 to 5 times followed by a gentle wash with distilled water. Then the seeds are sterilized in LAF (Laminar Air Flow) by using 70% alcohol for 1min and then washed with sterile distilled water for three times. Then the seeds were washed with 0.1% $HgCl_2$ for 10 min followed by 4 to 5 washes with sterile distilled water[7-8]. Then the surface sterilized seeds were inoculated in the media of different concentrations of heavy metals along with controls (1, 10, 20, 50, 100 ppm, T.W and D.W). All the inoculated tubes were transferred into a culture room at 25 ± 2^0C under 16 h light and 8 h dark conditions.

2.3 *Seedling growth and statistical analysis*

The data was collected after completion of 15 days of incubation period. The seedling morphology was observed including seed germination percentage, shoot and root lengths of the seedlings[7-8]. The percentage of germination was calculated by using the formulae i.e. number of seeds germinated among inoculated seeds. All the statistical work has been carried out using standard programs (excel-2007) in personal computer[7-8].

3. RESULTS AND DISCUSSION

Vigna mungo having high nutrient content was affected by different heavy metals such as Cr, Co, Cd and Pb in various regions globally. In the present work, *in vitro* seed germination and seedlings growth showed variations with different heavy metal treatments. Specifically, seedlings exhibited various modifications in physiological characteristics such as root and shoot growth by these heavy metal stresses. Cadmium is a highly toxic heavy metal that exhibits the decreased germination percentage from 1 ppm to 100 ppm (77% to 46%) (Figs. 1 and 2). Upon cadmium treatment, the seedling growth was markedly decreased from 1 ppm to 100 ppm i.e. shoot and root lengths when compared to controls (Figs. 3 and 4). Cadmium is dangerous to plant growth and ecosystems, once it enters into the plant through water and inhibits the root function i.e. water and ion uptake[13,19]. Cadmium stress makes the plant unable to control oxidative damage and regulates the root elongation, growth and possible reactions leads to cell death. Similarly in both *Vigna mungo* and *Phragmites australis*, the reduced growth was observed by Cd stress[20-21]. Schutzendubel and Polle[22] results also suggested the inhibition of growth by high concentration of cadmium. Keerthi Kumari et al[8,23] proved that high doses of cadmium inhibited seed germination in both sorghum and horse gram. Didwana and Bhalerao[24] also emphasized that higher concentration of cadmium reduces the seed germination as well seedling growth in black gram. The hexavalent chromium (Cr^{+6}) is responsible to produce active oxides in the cell and these oxides can combine with intracellular DNA leading to DNA damage by its cytotoxic effects[18,25]. In the present work, chromium stress demonstrated the negative impact on seed germination and seedling growth. Here 70% of germination was observed in controls and it was decreased to 36% in 100 ppm dose (Figs. 1 and 2). Overall, shoot and root lengths were also diminished at 100 ppm treatment (Figs. 3 and 4). But suddenly increasing of shoot and root lengths were noticed at 20 and 10 ppm. These results are in agreement with Sankar Ganesh et al [26] observations i.e. chromium inhibits the growth in paddy, black gram and soybean. They also demonstrated the reduction in germination percentage, root and shoot lengths because of accumulation of chromium in seeds. Similarly, Saminathan[27] proved that seed germination in five cultivars of black gram was reduced with increasing concentration of chromium. In the current work, the percentage of germination and seedling parameters including shoot and root lengths were altered after treatment with cobalt and lead. The germination levels were decreased more in cobalt treated *V. mungo* when compared to lead treated plants (Figs. 1 and 2). Specifically, seed germination percentage was reduced from 64 to 50 (from 1 to 100 ppm) in cobalt treatment, but in lead treatment the percentage of reduction was 78 to 70 only. This indicates that lead did not affect much on germination levels. Jayakumar and Vijayarengan[28] also found that high concentration of cobalt reduces the seed germination and seedling growth of black gram. In contrast, Abdul Jaleel[14] mentioned that low levels of cobalt promote the growth in *Zea mays*. Also, both root and shoot lengths were differently affected in both lead and cobalt treatments (Figs. 3 and 4). Kabir et al[29] and Farooqi et al[30] mentioned that lead treated *Thespesia populnea* and *Albizia lebbeck* seeds showed less germination and reduced seedling growth. Gangaiah et al[31] also observed differential growth with cobalt and lead independent treatments in pearl millet. Lead is one of the most noxious heavy metals which reduced the seed germination and also suppressed the growth by inhibiting the mitosis and causing extensive cytogenetic errors in black gram seedlings[32,33]. In the present study, we mainly noticed the decrease in root length by all the metals chosen (Cr, Cd, Pb and Co). In certain concentrations, there was an increase in germination including shoot and root lengths and this might be due to heavy metal critical points for black gram. Most of the heavy metals can lead to reduce the mitotic activity and persuade many types of chromosomal abnormalities which in turn inhibit the growth and development[34,35].

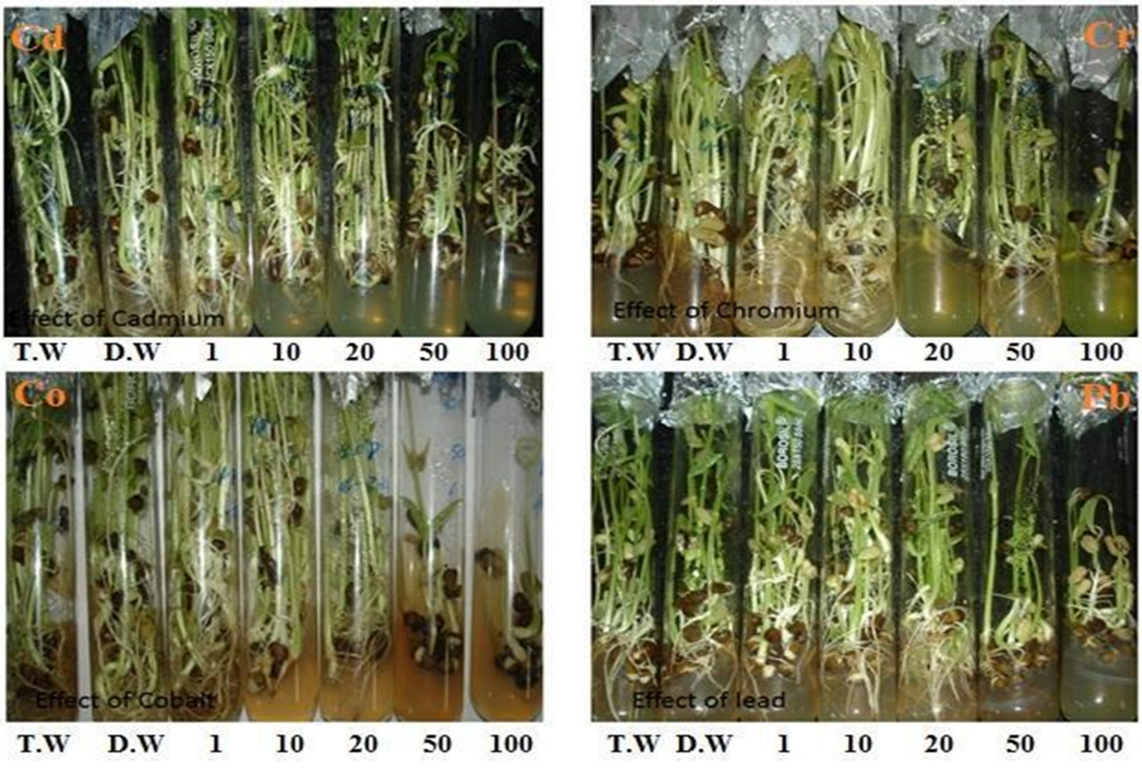

Fig 1: Effects of heavy metals on seed germination and seedling growth. T.W (tap water medium) and D.W. (distilled water medium) are the controls without any heavy metal. 1, 10, 20, 50 and 100 are the concentrations (in PPM) of the heavy metal.

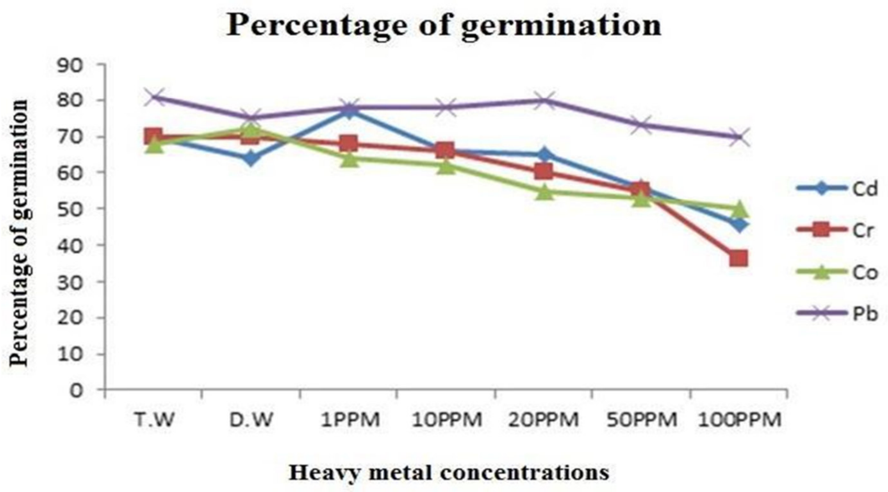

Fig 2: Effects of heavy metals on seed germination. T.W (tap water medium) and D.W. (distilled water medium) are the controls without any heavy metal. 1, 10, 20, 50 and 100 PPM are the concentrations of the heavy metal.

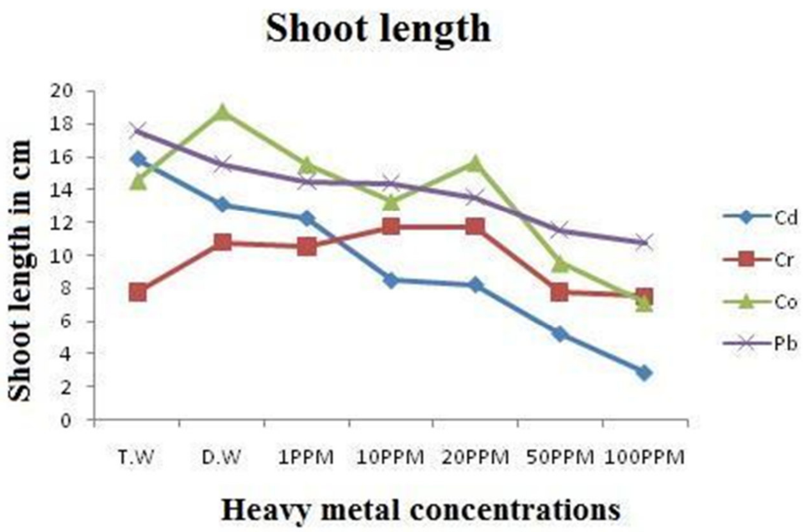

Fig 3: Effects of heavy metals on shoot length of the seedlings. T.W (tap water medium) and D.W. (distilled water medium) are the controls without any heavy metal. 1, 10, 20, 50 and 100 PPM are the concentrations of the heavy metal.

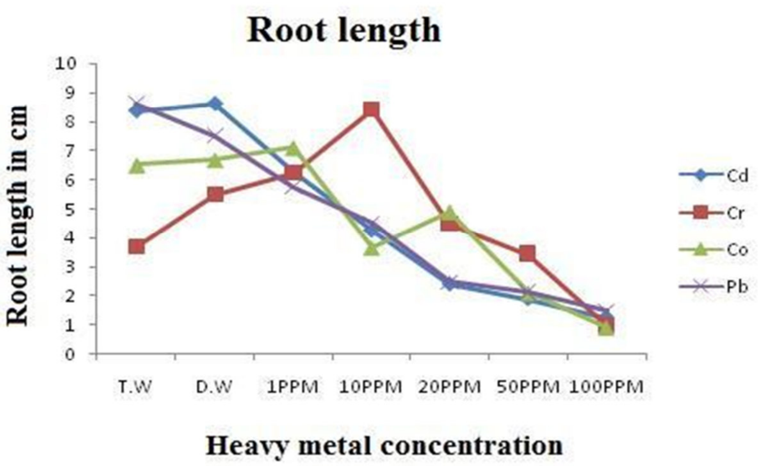

Fig 4: Effects of heavy metals on root length of the seedlings. T.W (tap water medium) and D.W. (distilled water medium) are the controls without any heavy metal. 1, 10, 20, 50 and 100 PPM are the concentrations of the heavy metal.

4. CONCLUSIONS

Black gram is one of the important food crops in certain regions worldwide but its yield was reduced drastically due to abiotic stress such as heavy metal pollution. In the current investigation, we found that all the four heavy metals (Cr, Cd, Pb and Co) exhibited a negative impact on growth of black gram. Percentage of seed germination was reduced from 1 ppm to 100 ppm in cadmium (77% to 46%), cobalt (64% to 50%) and lead (78% to 70%) treatments. Specifically, seed germination was drastically reduced (36% at 100ppm) in chromium treatment. Though lead and cadmium are highly toxic, here they show less toxic effect when compared to chromium specifically in germination aspect. The degree of response varies depending on the metal and concentrations. Also these data indicates that each metal responds differently in various crops. Present work may be useful for agricultural sector and also future research.

5. ACKNOWLEDGEMENTS

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6. AUTHORS CONTRIBUTION STATEMENT

In the present investigation T. Chandrasekhar, M.Bramhaiah and C. Madhava Reddy designed the work. M. Bramhaiah, M. Keerthi Kumari and T. Chandrasekhar performed the experiments. T. Chandrasekhar, L. Dakshayani, C. Madhava Reddy and S. Srinivasa Gowd wrote the manuscript and M. Subhosh Chandra and S. Adinarayana Reddy improved the manuscript with suggestions.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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