*Mycopath* (2012) 10(2): 71-76 **Research Article**

# *In vitro* toxicity evaluation of Cr(VI) against some pulses and their pathogen responsible for charcoal rot disease

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## Abstract

*In vitro* toxic influence of Cr(VI) was studied on seed germination and seedling growth of three pulse species namely mungbean [*Vigna radiata* (L.) Wilczek], mashbean [*Vigna mungo* (L.) Hepper], and cowpea [*Vigna unguiculata* (L.) Walp ]. Cr(VI) toxicity effect was also assessed on *Macrophhomina phaseolina* (Tassi) Goid, the cause charcoal rot disease in pulses and other field crops. Six different concentrations viz. 50, 100, 200, 300, 400 and 500 ppm of Cr(VI) were applied on seeds of the pulses in 9-cm diameter Petri plates lined with filter papers. All the six concentrations of Cr(VI) caused a significant reduction of 5–20%, 4–20% and 5–22% in germination, and root and shoot length, respectively. Higher concentrations (300–500 ppm) of metal solution caused more inhibition in investigated parameters as compared to lower concentrations (50-200 ppm). The sensitivity of the three pulses towards metal ions was found in order of: *V mungo > V. unguiculata > V. radiata.* Toxicity of Cr(VI) against *M. Phaseolina* was evaluated on 2% malt extract agar having nine Cr(VI) concentrations viz. 25, 50, 75, 100, 150, 200, 300, 400, and 500 ppm. Growth of the pathogenic fungus was markedly reduced by 20-90% at lower concentrations of 25 to 150 ppm and the fungus was unable to grow with further increasing metal concentrations. The present study concludes that early growth of three pulse species as well as their pathogen is very sensitive to Cr(VI). The contemporary research recommends for the elaborated investigation on involvement of plant pathogens and heavy metal on the growth and development of the economically important edible crops growing near industrial areas.

**Keywords:** Abiotic stress, Cr(VI), biotic stress, *Macrophomina phaseolina,* pulses.

## Introduction

Pulses are the dry edible seeds of third large economically important and highly nutritious flowering plants family Fabaceae or Leguminosae. In Pakistan, pulses occupy a significant place and serve as a vital source of protein and vitamins in diet of poor Pakistanis. Among different pulses grown, mungbean and mashbean are included in the major class, while cowpea is ranked amongst the minor group. These three pulses are widely cultivated as grain legumes, fodder and vegetable crops in many areas of Pakistan.

Over the past few decades the net production of pulses in the country is considerably suffering because of drastic influence of biotic and abiotic factors. Amongst the biotic factor, plant diseases caused significant loss (44%) to the pulses depending upon the crop variety (Bashir, 1988). It has been reported that mung and mash beans are attacked by about 26 diseases in the world (Charles, 1978; Tariq *et al*., 2006). Among the fungal diseases, charcoal rot is a common, widespread, destructive and economically important disease of cowpea, mungbean and mashbean under dry hot conditions. *Macrophomina phaseolina* (Tassi) Goid is the causal organism of charcoal rot that is soil and seed-borne pathogenic fungus; produces cushion shaped black sclerotia (Wheeler, 1975; Ahmad *et al*., 1991; Prabhu *et al*., 2012). This fungus caused seedling blight; stem rot, pod rot and wilting by blocking of xylem vessels and lead to the plant death (Abawi and Pastor-Corrales, 1990).

Abiotic stress due to heavy metal toxicity is one of the most critical factor needs to be stressed. Due to high cost and scarcity of chemical fertilizers the poor farmers have to use the agricultural, municipal and industrial wastes as a source of nutrients and organic matter (Younas and Shahzad, 1998). Unfortunately the abundantly consumed pulses have capability to uptake significant amount of contaminates particularly in the form of heavy metals from polluted soil (Bishnoi *et al*., 1993), that resulted in strong toxic effects (Nedelkoska and Doran, 2000). In this connection, literature showed significant reports on the toxicity of chromium (Cr) on different plant species (Jamal *et al.,* 2006; Li and Yang, 2006; Karbassi *et al.,* 2008). It has been reported that excess amount of Cr within the plant can cause stunted shoot growth and poor root development (Gbaruko and Friday, 2007). Cr lethality in plants also leads to leaves chlorosis, tissue necrosis, reduction in enzyme activity, photosynthesis and changing of chloroplast (Dube *et al*., 2003; Zayed and Terry, 2003; Scoccianti *et al*., 2006).

Previous literature focused more on the lethal effect of Cr(VI) on plants whereas metal poisoning on plant pathogens is still ignored. In metal-polluted soil plant pathogens may grow profoundly or may not able to grow. Nevertheless, under the influence of the adverse environmental effects the different types of the fungi became able to survive in the unfavorable conditions in the forms of scleroses, chlamydospores, or other (Agrios, 1997). There are some reports regarding effect of heavy metals on growth, amount of biomass, and enzymatic activity of fungi (Barabasz *et al*., 1997; Tkaczuk and Gorczyca, 2000). Therefore, it is necessary to be familiar with tolerance level of pathogenic fungi in metal-polluted soil to give recommendation for the management of plant diseases under metal stress environment.

Current study was focused to check the toxic effects of Cr(VI) at different concentrations on germination and seedling growth in *V. radiata, V. mungo* and *V. unguiculata* and on their pathogen *M. phaeolina*.

## Materials and Methods

Metal salt $K_2C_r2O_7$ (Merck) was used in whole experimentation trials. Different concentrations viz. 50, 100, 200, …… 500 ppm of metal solution were prepared by diluting stock solution with double distilled water.

Healthy seeds were sorted out and surface sterilized in by 0.1% sodium hypochlorite solution for 2-3 minutes and then rinsed 3-4 times with sterilized distilled water.

Method described by Li *et al*. (2006) was used with slight amendments to study the influence of various concentration of Cr by Petri plate experiment. Twenty surface sterilized seeds were sown on sterilized Petri plates (90-mm diameter) with single layer of filter paper (Whatman No. 42) wetted with 3 mL of metal solution. These Petri dishes were kept at 25 °C in the dark in growth cabinets for 4 days. Germination, and root and shoot length of germinated seeds were measured after 4 days.

The pathogen of charcoal rot disease i.e. *M. phaseolina* was procured from the Fungal Culture Bank, Institute of Agricultural Sciences, University of the Punjab, Lahore. Pure culture plates of the fungus were prepared on 2% malt extract agar (MEA) medium. The effect of Cr(VI) on growth of *M. phaeolina* was assessed according to protocol of Golubović-Ćurguz *et al*. (2010) with slight amendments. One hundred millilitres of MEA was prepared with addition of each of the nine concentrations of 25, 50, 75, 100, 150, 200, 300, 400, and 500 ppm of the metal solution. Pathogen inoculum was given, and Petri plates were incubated at 25 $^{o}$C. The effect of metal solution on growth of the fungus was checked after $7^{th}$ day of incubation. Control was taken without any addition of the metal solution. Growth inhibition of the fungal mycelial growth was recorded using the following formula.

$$\mathrm{MGI}(\%) = \frac{\mathrm{DC - DT}}{\mathrm{DT}} \times 100$$

dt = Diameter (cm) of the fungal colony in control; de (cm) = diameter of the fungal colony in metal solution treatments.

Duncan's Multiple Range test (P≤0.05) was used to delineate treatment mean (Steel *et al*., 1980), using computer software COSTAT.

## Results

### Seed germination under Cr(VI) stress

There was significant reduction of 5–20% in germination of *V. mungo*, *V. radiata* and *V. unguiculata* due to lower concentrations (50 to 300 ppm) of Cr(VI) over control. However, higher concentrations (400 & 500 ppm) caused the maximum reduction of 10% and 20%, respectively, in germination of all the three pulses when compared with control (Fig. l).

### Root length under Cr(VI) stress

There was a reduction of 4–20% in root length of the three pulse species. The highest reduction of 5–20% in root length was recorded in case of *V. radiata* followed by 5–18% in *V. unguiculata* and 4–15% in *V. mungo* on application of Cr(VI) concentration ranging between 50–500 ppm (Fig. 1) .

### Shoot length under Cr(VI) stress

Shoot length in the three pulses was significantly declined by 5–22% due to various treatments of Cr(VI) over control. The highest inhibitory influence of the metal solution was recorded on *V. unguiculata* shoot in comparison with rest of two pulses. In *V. unguiculata* shoot length was significantly reduced by 4–10% and 16–22% due to metal concentrations of 50–200 ppm and 300–500 ppm, respectively. In rest of *Vigna* species (*V. radiata* and *V. mungo*), there was significant reduction of 4–10% in shoot length at low concentrations of 50–200 ppm and a decline of 15–19% at 300–500 ppm, over control (Fig. 1).

**Influence of Cr(VI) on the growth of pathogen: *M. phaseolina***

There was significant suppression (20–100%) in growth of the pathogenic fungus due to nine different concentrations of Cr(VI). However, metal solution applied at higher concentration range of 200–500 ppm completely arrested the surface mycelial growth of the fungus (Fig. 2).

## Discussion

Presently, different concentrations of Cr(VI) exhibited significant reduction in seed germination, and shoot and root length in the three pulse species in Petri plates. Cr(VI) treatments at higher concentrations (300–500 ppm) found to be responsible for the highest reduction in seed germination and seedling growth over low concentrations (50–200 ppm). The inhibition of seedling growth by Cr could be mainly due to the accumulation of Cr in the roots. Bishoni (1993) observed that hexavalent Cr applied as potassium dichromate did not affect the percentage germination of pea seed but the growth of radicles and plumule were significantly suppressed above 0.5 mM concentration. Similarly, Kopittke *et al*. (2007) noticed poisonous influence of Pb on cowpea and stated that root is primary site of metal toxicity, a consequence of impaired root growth and function reduced shoot growth. Supplementary rationale for decline in shoot and seedling length of the three pulses could be probably owing to the decrease in activity of meristematic cells present in this region and some enzymes contained in the cotyledon and endosperm cells. The enzymes in cotyledon and endosperm cells may become active and begin to digest stored food which converts to soluble form and is transported to radical and plumule tips. So when enzymatic activities were affected, the food did not reach to radical and plumule and in this way shoot and seedling length were affected (Kabir *et al*., 2011). According to Mahmood *et al*. (2007), metal induced changes in the seed metabolic processes followed by structural and morphological properties of the roots are possibly related to a substantial decrease in metal tolerance ability of the crop seedlings.

Among the three pulse species, *V. radiata* and *V. unguiculata* were found to be more sensitive to detrimental influence of Cr(VI) than *V mungo*. Various degrees of permeability of seed coats to metals lead to a range of seed germination inhibitions (Wierzbicka and Obidziniska, 1988).

In the current work, nine different concentration of Cr(VI) produced very toxic influence on growth of *M. phaseolina*. The fungus was unable to grow at higher concentrations of metal (Tkaczuk *et al.,* 1997). It has been documented previously that heavy metal ions strongly inhibited surface growth of fungi. The intensity of the response depends on the fungal strain as well the metal ions and their concentration (Popowska-Nowak *et al.,* 2000; Ropek and Para, 2003; Golubović-Ćurguz *et al*., 2010). Jaworska and Gorzyca (2004) reported strong poisonous influence of Cu, Cr and Zn on the growth of *Paecilomyces fumosoroseus.* Ropek and Para (2003) reported that Cu and Pb drastically reduced growth and sporulation of *P. farinosus*. Similarly, Tkaczuk (2005) found that higher concentration of Ni, Cu, Zn and Cr, added to the media prevented the growth of the pathogenic fungi.

## Conclusion

Different Cr(VI) treatments induced toxic influence on germination and seedling growth of *V. radiata, V. mungo* and *V. unguiculata.* The overall inhibitory effect of the metal solution was more pronounced on *V. mungo* and *V. unguiculata* than on *V. radiata.* The pathogenic fungus *M. phaseolina* also exhibited the highest degree of sensitivity to the presence of Cr(VI) ions. The combined influence of the pathogen and the metal ions could be restrictive factors for the growth and development of plant especially for palatable crops growing in contaminated soil. Thus, there is urgent need to address this emerging threat to plants due to combine action of biotic and abiotic stresses.

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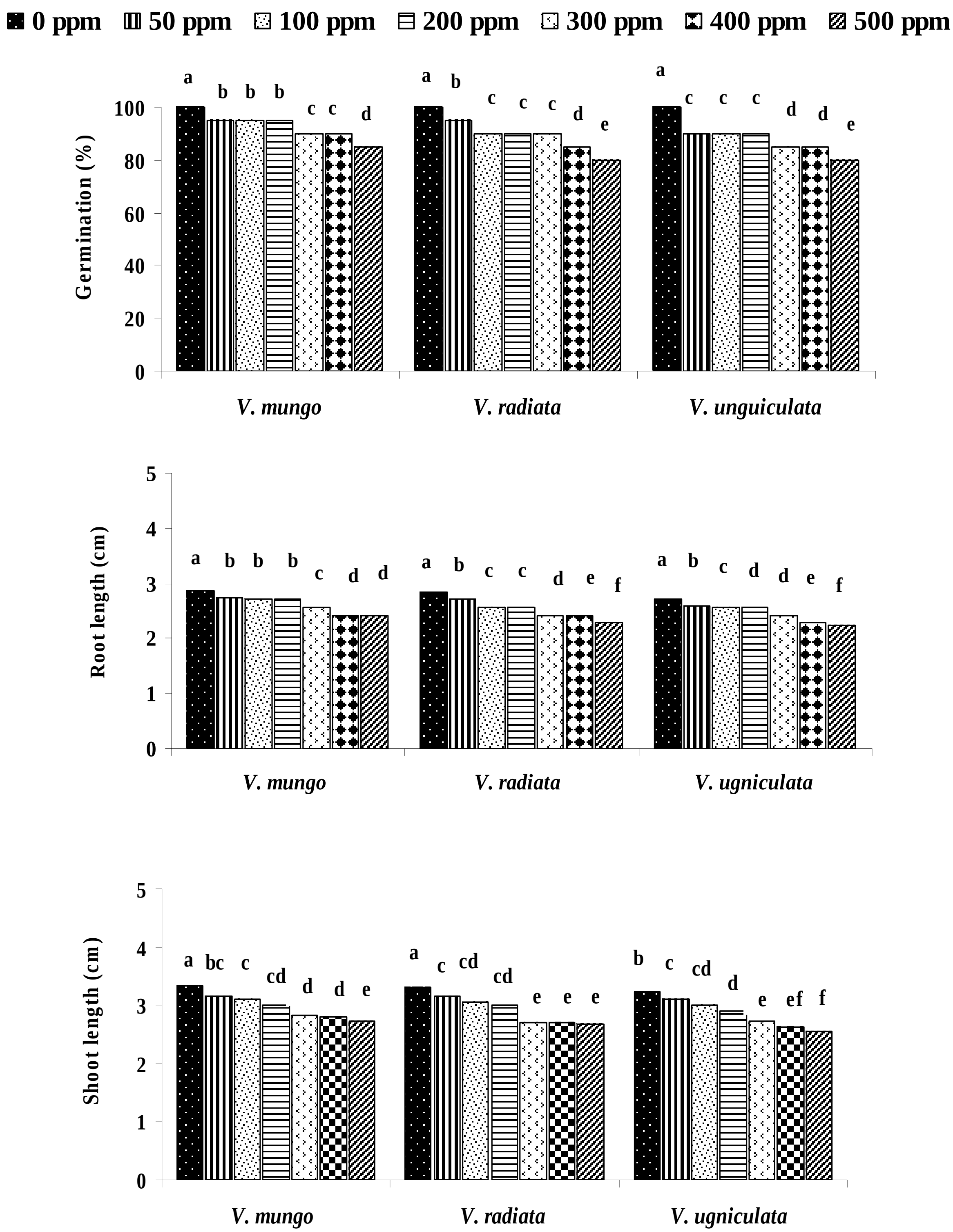


**Fig. 1:** Influence of different concentration of Cr(VI) on seed and seedling growth of three pulse species**.** Values with the same superscript letters are not significantly different among treatments at $P \leq 0.05$ according to Duncan's Multiple Range tests.

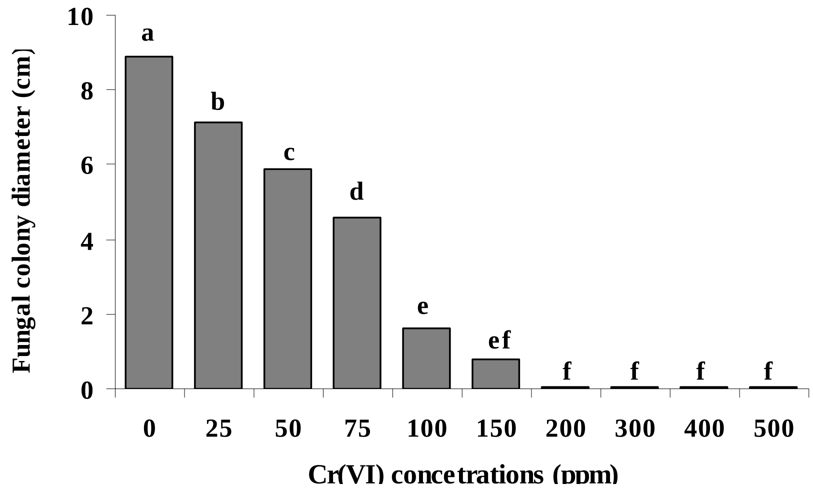


**Fig. 2:** Effect of different concentrations of Cr(VI) on growth of *Macrophhomina phaseolina.* Values with the same letters are not significantly different at P ≤ 0.05 according to Duncan's Multiple Range test.


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