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Growth of *Vigna radiata*, *V. mungo* and *V. unguiculata* under abiotic stress of mercury

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Abstract

Mercury (Hg) poisoning in soil has become a global issue due to increase demand of safe food. In present study, toxic influence of Hg(II) was checked on seed and seedling growth of three pulses namely *Vigna radiata* (L.) Wilczek, *V. mungo* L. Hepper and *V. unguiculata* (L.) Walp both in vitro and in vivo conditions. In laboratory bioassays, pulse seeds were exposed to 3 mL of six different concentrations (50-500 mg L^{-1}) of Hg(II) in 9 cm diameter Petri plates. Germination percentage, shoot and root significantly declined by 2-30% in all *Vigna* spp. after 96 hours of growth over control in Petri plates. In pots, Hg(II) was applied in similar six concentrations in 360 g of soil. The germination rate, shoot and root growth were significantly reduced by 5-30%, 8-28% and 10-35% due to increasing concentration of Hg(II) in soil in 15-days old seedling over control. Morphological symptoms like wilting and necrosis of leaf margins, disorientation of roots and shoots growth were observed at higher dose of Hg(II). *V. unguiculata* and V. *radiata* were sensitive to detrimental influence of Hg(II) than *V. mungo*. In conclusion, Hg(II) had significant drastic effects on early growth of pulses, therefore more research is needed to explore the toxicity of different mercury based pesticides and fungicides that are generally utilized on agricultural land to increase yield and profit of crop.

Keywords: Pulses, abiotic stress, Hg(II) biotic stress, *Macrophomina Phaseolina*.

Introduction

Mercury (Hg) is one of the main contaminant in soils because of the annual incorporation into the agricultural lands (Patra and Sharma, 2000). It is a highly phytotoxic, nonessential, non-biodegradable and immutable metal (Nayna *et al*., 2005) that is added into the agricultural land through application of sludge, fertilizers, lime, and manures (McLaughlin *et al*., 1996). The vital source of contaminating agricultural soil is exploitation of organic mercurials as a seed-coat dressing to prevent fungal diseases in seeds (Patra and Sharma, 2000). Rashid and Mukherji (1993) reported decrease in different growth parameters and yield of wheat due to foliar applications of lead nitrate solution. However, exceding levels of mercury induce adverse abnormalities in plant biochemical and physiological processes along with alteration of translocation of minerals (Gupta *et al*., 1998; Srivastava *et al*., 2005; Moreno-Jimenez *et al*., 2007). Abscission of older leaves, reduction in growth, vigour, chlorophyll content and nitrate reductase activity have been documented as a result of rigorous Hg poisoning in plants (Vyas and Puranik, 1993). Significant reduction plant growth, biomass induces disorientation of roots and shoots, plant tissue, and finally the cell wall (Sharma *et al*., 2000; Patra and Sharma, 2000). It has been documented that higher concentrations (> 1-2 mg L^{-1}) of Hg drastically declined the growth of tomato (Cho and Park, 2000), wheat (Liu *et al*., 2010) and cow pea (Abdul Hamid *et al*., 2011).

Pluses are plants of family Leguminosae have capability to uptake specific heavy metals from polluted soil (Bishnoi *et al*., 1993). Plants probably growing in metalliferous habitats have the capacity to inactivate the metals via the binding of metal ions and by changing the chemical composition and physical organization of their cell membranes (Stefanov *et al*., 1995). The highest risk for human health is when plants develop tolerance mechanisms against metals and when those plants are incorporated into the food chain. Growth changes are the first most obvious reactions of plants under stress (Breckle *et al*., 1991). Therefore, seed and seedling growth of plant are very imperative parameters

that may harmed by stressed conditions (Shah and Dubey, 1995) like heavy metals results are just not yield losses but shifting of these pollutants through the food chain to living beings. In Pakistan, crop contaminations due to the heavy metals toxicity have received a very limited attention (Mahmood *et al*., 2007).

Therefore, current study was focused to check the toxic effects of different concentrations of Hg(II) on the seed germination, hypocotyls and root growth in *V. radiata*, *V. mungo* and *V. unguiculata* both in Petri plates and pots.

Materials and Methods

Seeds of three *Vigna* spp. were obtained from Punjab Seed Cooperation, Lahore. Pakistan. Healthy, homogenous seeds were surface sterilized with 3% H_2O_2 for 15 min followed by repeated washing with distilled water.

The stock solutions of Hg(II) were prepared by dissolving measured amount of $HgCl_2$ in one liter of distilled water. Further dilutions were made from the stock solution with double distilled water.

Petri plate bioassay studies were carried out following the method of Li and Yang (2006). Twenty seeds of each of three pulse species were placed in sterilized Petri plates (9cm x 2cm) having sterilized filter paper (Whatman No.1), moistened with 3 mL of metal solution. The same procedure was performed for each of six different concentrations (50, 100, 200, 300, 400 and 500 mg L^{-1}) of Hg. Petri plates were incubated for 96 hours at room temperature (27^oC; 16 h photoperiod). Seeds treated with 3 mL of distilled water acts as control. The experimental design was a randomized complete block with five replicates for each.

The germination, root and shoot lengths of germinated seeds were measured after 4^{th} day of incubation. The root length was determined by radicle formation of germinated seeds. Germination percentages in Hg treatment groups were calculated using Equation

$$\text{Germination (\%)} = \frac{\text{Germinated seeds}}{\text{Total seeds}} \times 100$$

Protocol given by Park *et al*. (2008) was followed with some modification for assessing the influence of Hg(II) on seed and seedling growth of elected pulses in pots. Initially twenty surface sterilized and metal imbibed seeds per pots (6cm x 10 cm) filled with sandy loam soil (350 g) were sown at the depth of 1cm. After germination, thinning was done to maintain six uniform seedlings in each pot. 50 mL of metal ($HgCl_2$) solution in different concentration was applied in each pot only at first day of sowing. While, for the rest of days each pot was treated with distilled water. Plants received only distilled water acts as control. Pots were arranged in a completely randomized design in natural environmental conditions. Germination rate of seeds were recorded carefully after 4 days of sowing. Growth parameters were recorded after 15^{th} day of seedling growth.

Data regarding the various parameters of seed germination and growth was analyzed by applying Duncan's Multiple Range Test (Steel & Torrie, 1980)

Results

Generally, a significant reduction in germination, root and shoot growth parameters was observed at higher concentrations (300, 400 and 500 mg L^{-1}) of Hg(II) than lower concentrations (50, 100 and 200 mg L^{-1}) and control treatments both in Pertiplates and pots.

In pertiplates, germination rate both in *V. radiata* and *V. unguiculata* was significantly suppressed by 5-15% due to lower concentrations (50-200 mg L^{-1}) and by 15-25% due to higher concentrations (300-500 mg L^{-1}) of metal over control. In *V. mungo*, there was 10-20% reduction in germination rate due to different concentration of metal over control (Fig. lA). Shoot length was significantly reduced by 3-26% both in *V. ungniculata* and *V. radiata* and by 5-21% in *V. mungo* with increasing concentration of Hg(II) over control (Fig. 1B). There was gross decline of 12-30% in root growth of *V. radiata* followed by 10-26% in *V. unguiculata* and 9-19% in *V. mungo* with rising concentrations of Hg(II) as compared to control (Fig. 1C).

Treatment of pots with six concentrations of 50, 100, 200, 300, 400 and 500 mg L^{-1} resulted in a significant reduction of 10-30%, 10-25% and 10-22% in germination rate and shoot growth of *V. ungiculata*, *V. radiata* and *V.*

mungo in comparison to control (Fig. 2A & 2B). The increases in metal concentration not only decreased the germination rate but also delayed the germination initiation. Morphological symptoms like wilting and necrosis of leaf margins, disorientation of shoots and roots growth were observed at higher dose (400 and 500 mg L^{-1}) of Hg(II). Root growth showed more sensitivity to higher concentration of Hg(II) as compared to germination rate and shoot growth. Metal amended soil exhibited a parallel and significant decline of 12-35% in root growth of *V. unguiculata* and *V. radiata* and 9-21% in *V. mungo* as compared to untreated soil (Fig. 2C).

Discussion

Mercury was found to be very harmful to growth of three pulses particularly at higher concentrations. There was a net reduction of 5-35% in seed germination, shoot and root length of three test plants both *in vitro* and *in vivo* conditions. These results are in concordance with present findings showing that in most cases Hg, with no known beneficial function induced the highest toxicity in plants (Munzuroglu and Geckil, 2002). Likewise, it has been reported earlier that some heavy metals at low doses are essential micronutrients for plants but in higher doses they may cause metabolic disorder and growth inhibitor for most of the plant species (Ferandes and Henriques, 1991). Reduction and delayed seed germination rate of three pulses provided evidence that excessive amount of Hg could impart negative effects on water uptake and water movement of plant (Poschenreider *et al*., 1986). Decline in water absorption and transport along with water stress tolerance (Barcelo *et al.,* 1988) resulting in lower plant growth and development.

The reduction in growth of shoot and root might be due to reduction in cell division, deleterious effect of Hg(II) on photosynthesis, respiration and protein synthesis (Vijayaragavan *et al*., 2011). The overall negative influence metal on plant resulted in the retardation of normal growth (Kupper *et al.,* 1996) with adverse abnormalities in plant morphology. In addition, metal toxicity caused decrease in root elongation and root formation, root tip damage, inhibition in growth rate of elongation cells as reported by different workers (Hagemeyer and Breckle, 2002; Marcnano *et al.,* 2002). Kopittke *et al*. (2007) found root as the main site of Pb(II) toxicity, with the reduced shoot growth a consequence of impaired root growth and function.

Root growth in three pulses found more sensitive to than shoots. It has been suggested that the accumulation of metal in the roots occurs because the endodermis functions as a barrier to the radial transport of metal in the root, thereby restricting its movement to the shoots (Seregin and Ivanov, 1997).

Among three pulse species, *V. unguiculata* were found to be more sensitive to detrimental influence of Hg(II). Similar to present findings results were obtained by Mahmood *et al*. (2007), while evaluating toxic influence of Pb and Zn on different cereal species. A lack of consistent adverse effects exerted by Hg(II) on seed germination is most probably related to interspecies differences in seed coat structures for regulating metal absorption. It is reported that plant seeds have inherent capability for selective absorption of metals in nature (Stefanov *et al*., 1995). This suggests that seed coat structures of *V. radiata* and *V. unguiculata* and *V. mungo* may have selectively reduced Hg(II) absorption from the solution to minimize their adverse effects on germination and seedling growth.

Conclusion

It is appeared from the current investigation that increasing concentrations (50-500 mg L^{-1}), of Hg(II) impart drastic influence on the germination and seedling growth of *V. radiata*, *V. mungo* and *V. unguiculata*. The toxic influence of metal was more severe on *V. unguiculata* followed by *V. radiata* and *V. mungo*. This might be an indicator of sensitivity of plant early development to Hg(II). In future more research should be focused on growth and development of plant cultivated in unregulated waste amended agricultural soils to avoid from future food security problems.

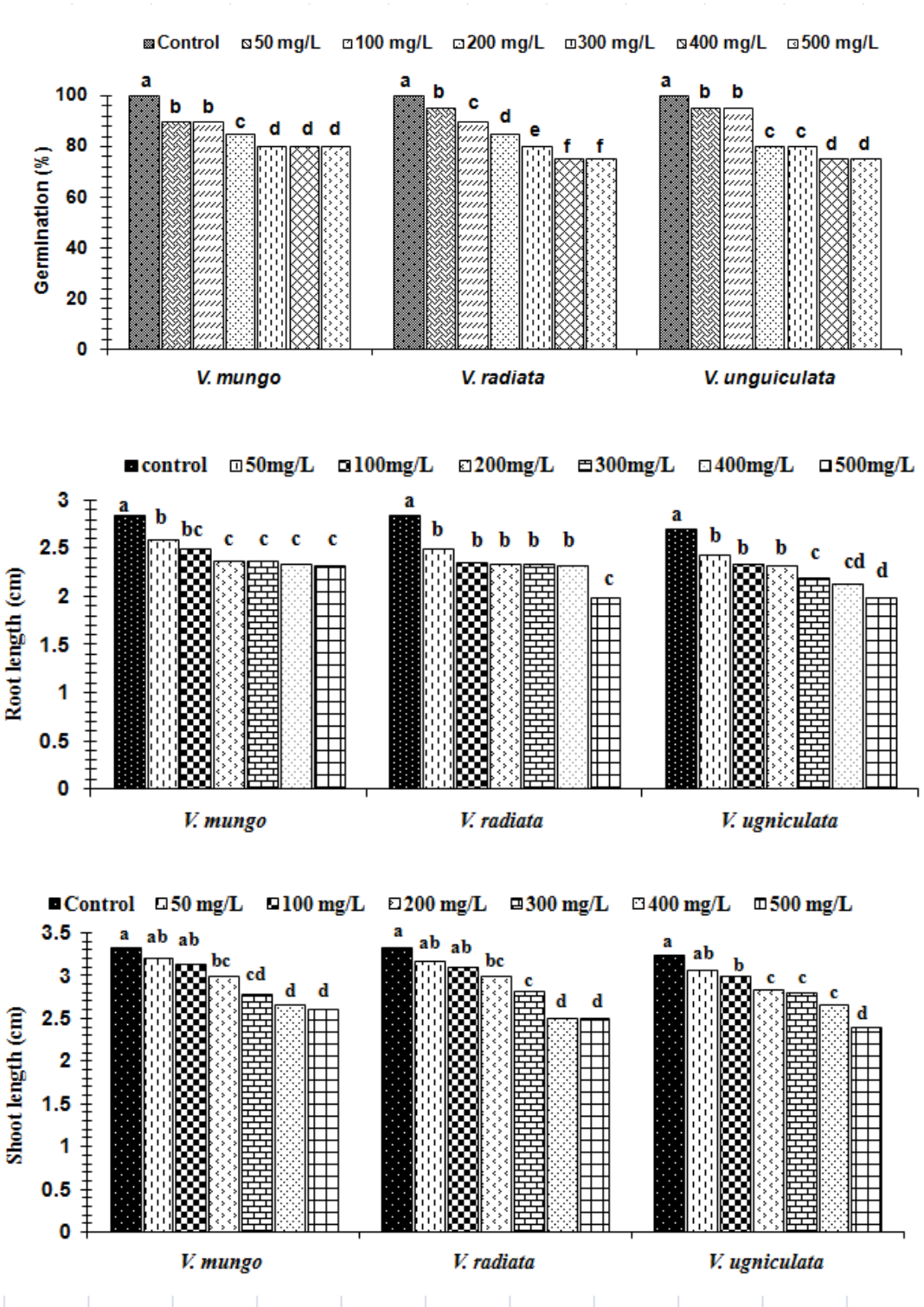

Fig 1. *In vitro* influence of different concentrations of Hg(II) on seed and seedling growth of three pulse species. Values with the same superscript letters are not significantly different among treatments at $p < 0.05$ according to Duncan's multiple range tests.

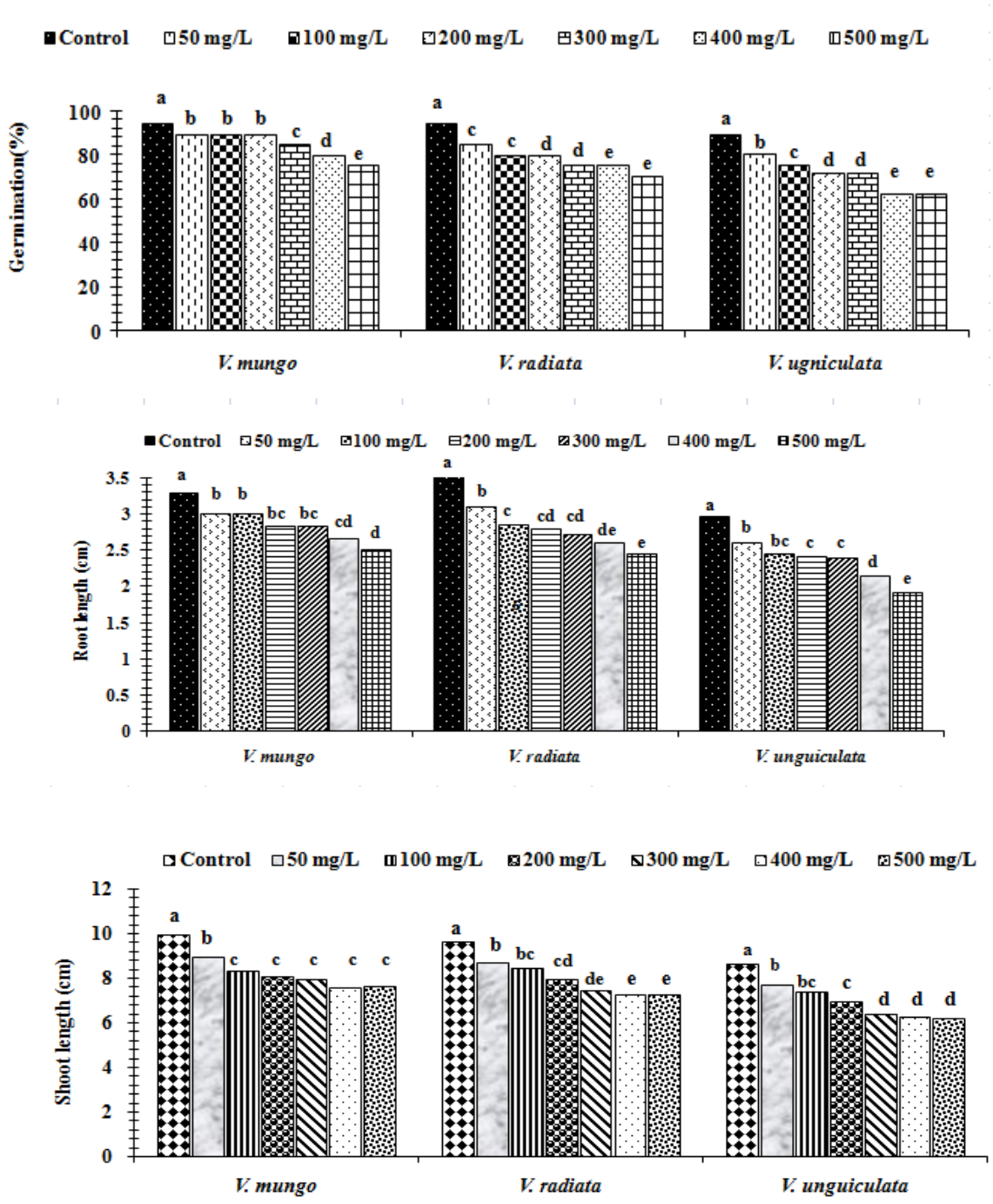

Fig 2. *In vivo* influence of different concentrations of Hg(II) on seed and seedling growth of three pulse species. Values with the same superscript letters are not significantly different among treatments at $p < 0.05$ according to Duncan's multiple range tests.

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