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Physiological and Morphological Responses of *Phaseolus vulgaris* Caused by Mercury Stress under Lab Conditions

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Physiological and Morphological Responses of *Phaseolus vulgaris* Caused by Mercury Stress under Lab Conditions

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Abstract
One of the many new risks that effecting the early societies is the continuous exposure to pollutants, namely, heavy metals. Mercury (Hg) is perhaps the metal which has attracted the most attention in soil science and plant nutrition due to its potential toxicity to ecosystem. In the present study, the toxic effect of mercury was determined by morphological and physiological parameter on plant *Phaseolus vulgaris.* In germination studies, parameters such as germination percentage, root length, and shoot length were decreased with increasing dose of mercuric chloride ($HgCl_2$) concentrations. Mercury also showed inhibition property towards physiological parameters such as chlorophyll, protein, nitrate, and endogenous pool. Higher concentrations of $HgCl_2$ were found to be more toxic.

Keywords: Mercury; *Phaseolus vulgaris*; Nitrate reductase; Endogenous pool.

1. INTRODUCTION

Toxics Link in India has been involved both at the global and the national level in working on the issues of mercury (Hg). Hg distribution in the environment has been a focus of scientific attention because of the potential health risks posed by Hg exposure (Sahni, 2011). Anaerobic organism's bio-transform the inorganic form to methyl mercury which gets bio-accumulated in food chain. It is the most toxic form of Hg (Azevedo and Rodriguez, 2012). The influence of metals on development and reproduction of plants can be firstly quantified by determining the germination traits of seed and growth performance of seedling (Patra and Sharma, 2000). Hg is a strong phytotoxic as well as genotoxic metal (Fridovich, 1986; Suszcynsky and Shann, 1995).

The harmful effect of Hg in the plants has been studied in several plant species: pea (*Pisum sativum* L.), ryegrass (*Lolium perenne*), spearmint (*Mentha spicata* L.), Norway spruce (*Picea abies* L.), spinach (*Spinacia oleracea*), and willow (*Salix*) (Beauford *et al.*, 1977; Al-Attar *et al.*, 1988; Godbold and Hüttermann, 1988; Chunilall *et al.*, 2004; Du *et al.*, 2005). Adverse effects caused by excessive Hg include membrane structure integrity disruption (Chengcang, 1998) mineral nutrient uptake reduction (Cho and Park, 2000; Patra and Sharma, 2000), photosynthesis, and transpiration reduction (Krupa and Baszynski, 1995).

Hg in the plant's regime is still not fully known. The information about growth inhibition or toxic mechanism in plant is still known less. *Phaseolus vulgaris*, an important legume crop and a great source of nutrition to millions of people. The effect of Hg on morphological and physiological characteristics of *P. vulgaris* has not been studied yet. The objectives of the present study were: (1) to investigate the effects of Hg on the growth parameters like shoot, root length, and seed germination (2) to evaluate the effects of Hg on nitrate activity (*in vivo*). The results should be helpful to clarify the potential elucidation of Hg detoxification mechanisms in *P. vulgaris.* It will correlate the nitrogen static of the plant with growth and yield of crop.

2. MATERIALS AND METHODS

2.1. Seed Material

For the present study rajma seeds were collected from a local market. Bean seeds were sterilized by 0.1% mercuric chloride ($HgCl_2$) for 30 sec and then washed properly with three time autoclaved double distilled water.

2.2. Experimental Design

River sand was washed and sterilized properly. Firstly sand was washed with normal water then followed by 24 h acid treatment, and finally washed with water thrice. Each pot had 1 kg sand with a cast of 20 seeds. Pots were placed in continuous light 30 wm^{-2} intensity supplied by fluorescent tubes at 26 ± 2ºC for 7-8 days. Half strength Hoagland solution without nitrogen was used for watering (Hoagland and Arnon, 1938).

2.3. Test Chemicals

$HgCl_2$ was used as test chemical. Different concentrations of the compounds were prepared (namely 0, 0.001, 0.01, 0.1, and 1 mM) using half strength Hoagland solution as solvent.

2.4. Metal Treatment

Metal treatment was given in different schedule.

- For morphological study—acid wash sand treated with different concentration of Hg.
- For physiological study—primary leaves form uniformly grown seedling were used for different treatments.

2.4.1. Morphological assay: For this study, acid washed sand was used. The pH of sand was 6.2. Then in each pot 1 kg of sand was treated with different concentration of Hg for 24 h. Followed by the transfer of 20 properly sterilized and washed rajma seeds into each pot. For regular watering Hoagland solution was used. After a week seedlings were harvested then root and shoot were separated for measurements. Seed germination was also counted. All values counted in three replicates of experiment (Ling *et al.*, 2010; Sharma *et al.*, 2009).

2.4.2. Physiological assay: For this study, first excised bean leaf (0.25 g) were cut into small pieces treated with different concentration of $HgCl_2$ for 24 h incubation in continuous light inside "Indosan growth chamber" BOD for 24 h. The leaf is then used in estimation of nitrate reductase, endogenous nitrate pool, protein, and chlorophyll.

2.4.3. Nitrate reductase assay: The activity of nitrate reductase in the treated material was estimated by *in vivo* (Srivastava, 1974) method with slight modification. About 0.25 g of leaf material were incubated with 10 ml of incubation medium consisting of 0.1 M sodium phosphate buffer (pH 7.2), 0.2 M KNO_3, and 25% isopropanol in dark vial of 20 ml capacity. The whole set was incubated in dark for 30 min at 30°C. Nitrite released in the incubation mixture due to enzyme activity was measured by color development by the formation of diazo compound with sulfanilamide and nitrate coupled with NED to give a red dye. The absorbance was read at 540 nm after 20 min by using UV-spectrophotometer. In intact seedlings nitrate reductase activity was measured only in the leaves as they are believed to be major nitrate reducing organic in most plants. Endogenous nitrate pool in the leaf segments were estimated according to Aslam (1981).

2.4.4. Pigment identification: For chlorophyll estimation primary treated leaves 0.25 g were extracted with 80% acetone. The extract was centrifuged at 5000 rpm for 10 min. Absorbance was calculated at 663 and 645 nm. Total chlorophyll contained was calculated by method of Strain and Svec (1966). Total protein was determined by Lowry *et al.* (1951). The protein in the enzyme solution was precipitated out by 10% TCA followed by solubilization with NaOH. Absorbance was measured at 660 nm using double beam spectrophotometer.

2.5. Statistical Analysis

Each experiment was repeated at least thrice and data presented are the average value and standard deviation value of findings. Statistical data collected from one-way ANOVA test software.

3. RESULTS

3.1. Effect on Germination, Shoot, and Root Length

The highest percentage of seed germination was observed in control then continuously decreased. In 1 mM there was only 30% germination (Table 1).

As the concentration of $HgCl_2$ increases there was retardation in growth period of beans. As shown in Table 2. There was increase in growth days of 0.1 and 1 mM concentration in comparison to control (without treatment). Value in terms of mean and standard deviation (SD).

Table 1: Effect of $HgCl_2$ on germination of *P. vulgaris* seed.

Concentration of $HgCl_2$ (mM)	Germination (%)
Control (without treatment)	60
0.001	53.33
0.01	40
0.1	35
1	30

Table 2: Decline in periods of seed germination.

Concentration of $HgCl_2$ (mM)	Growth in days (Mean ± SD)
Control (without treatment)	7.33 ± 2.08
0.001	10.00 ± 4.36
0.01	12.67 ± 4.04
0.1	12.33 ± 3.79
1	13.33 ± 3.06

Table 3: Effect of $HgCl_2$ on root and shoot lengths.

Concentration of $HgCl_2$ (mM)	Shoot length (cm)	Root length (cm)
Control (without treatment)	11.53 ± 2.637	6.861 ± 3.166
0.001	10.44 ± 11.52	4.75 ± 1.746
0.01	11.52 ± 2.219	5.042 ± 202.1
0.1	10.98 ± 2.492	6.262 ± 2.672
1	10.11 ± 1.787	3.111 ± 0.814

At end of experiment root and shoot lengths were measured by ruler in cm. There was little mean difference from control to 1 mM concentration in shoot length but there was more inhibition shown in root length from control to 1 mM treated set (Table 3). The values were shown in average mean or in terms of SD.

3.2. Effect on Physiological Assay

The effect of Hg on nitrate depend on concentration or whether the experiment was *in vivo* or endogenous (Figures 1 and 2). In endogenous nitrate pool there was a continuous inhibition from 0.001 to 1 mM or significant decrease in 0.1 and 1 mM concentration. Hg shows inhibition in endogenous pool with the increased concentration however in *in vivo* nitrate assay there was least inhibition in 1 mM and increased in 0.001 mM as shown in Table 4.

Figure 1: Inhibition in nitrate reductase concentration with respect to $HgCl_2$.

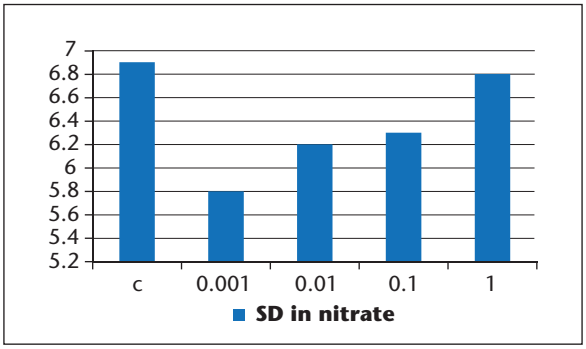

Figure 2: Inhibition in endogenous nitrate pool concentration with respect to $HgCl_2$.

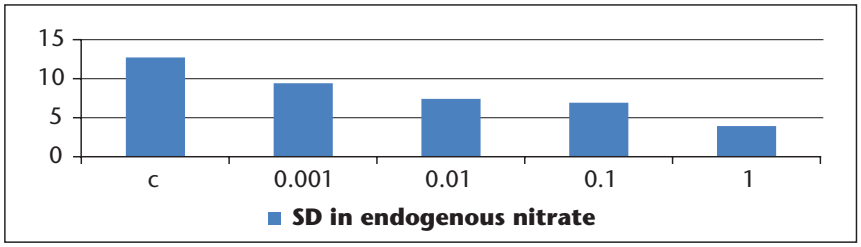

Table 4: Effect of Hg on nitrate *in vivo* and endogenous pool in excised leaves.

Concentration of $HgCl_2$ (mM)	Nitrate reductase inhibition (*in vivo*) (µ mol NO_2/hr/g fresh weight) Mean ± SD	Endogenous nitrate pool (µ mol NO_2/hr/g) fresh weight) Mean ± SD
Control (without treatment)	6.9 ± 1.8	12.78 ± 2.30
0.001	5.8 ± 2.9	9.43 ± 3.16
0.01	6.2 ± 2.8	7.43 ± 2.87
0.1	6.3 ± 2.3	6.95 ± 3.10*
1	6.8 ± 2.3	3.99 ± 3.56**

**$p < 0.001$ and *$p < 0.01$.

Figure 3: Inhibition in chlorophyll concentration with respect to $HgCl_2$.

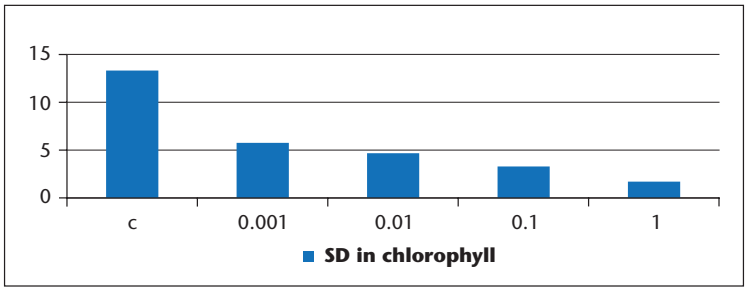

Figure 4: Inhibition in protein concentration with respect to $HgCl_2$.

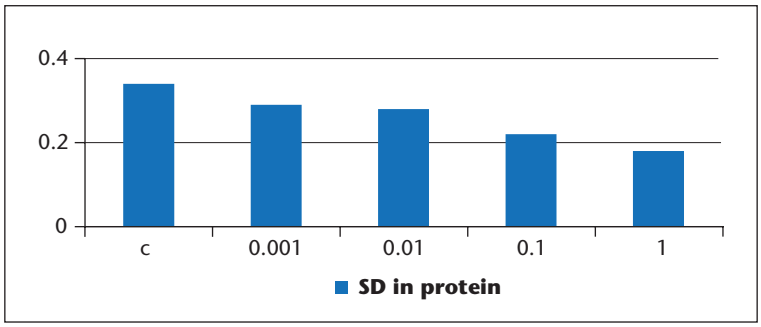

Table 5: Effect of Hg on chlorophyll and protein in excised leaves.

Concentration of $HgCl_2$ (mM)	Cholorophyll (mg/g fresh weight) Mean ± SD	Protein (mg/g fresh weight) Mean ± SD
Control (without $HgCl_2$)	13.48 ± 8.0	0.34 ± 0.09
0.001	5.81 ± 2.5	0.29 ± 0.11
0.01	4.74 ± 1.5**	0.28 ± 0.07
0.1	3.30 ± 1.4**	0.22 ± 0.04
1	1.75 ± 0.99**	0.18 ± 0.06*

**$p < 0.001$ and *$p < 0.01$.

The effect of Hg on chlorophyll and protein was increased with increased concentrations. Chlorophyll estimation showed a proper inhibition with increased concentration of Hg, high inhibition at 1 mM shows $p < 0.01$ significance then followed by 0.1, 0.01 mM concentration with same significance $p < 0.01$ (Figure 3). In protein estimation the value was continuously decreased from 0.001 to 1 mM concentration (Figure 4) as shown in Table 5.

4. DISCUSSION

Phytotoxic effects of heavy metal ions have been widely reported. The morphological and physiological aspects of metal toxicity, however, have been explored only in a few processes. The relatively strong affinities of heavy metal ions for side chain ligands of proteins indicate that enzyme activities and other functional proteins are one of the primary targets of metal toxicity (Vallee and Ulmer 1972; Hampp *et al.*, 1976).

The visible toxicity symptoms of $HgCl_2$ treatment was stunting and chlorosis, browning of leaf tip, reduction in growth, and stunting of seedlings and root were the morphological symptoms of Hg toxicity. The inhibition of root growth and development of lateral roots are symptoms of toxicity due to Hg which can be attributed in the inhibition of mitosis, reduced synthesis of cell wall components and changes in photosynthetic activity (Patnaik and Mohanty, 2013). The involvement of phytochelatin (PC) synthesis due to Hg adaptive response (Sharma and Subhadra, 2010). Similar observations with Hg treatment to phaseolus seedlings were noticed by Mor *et al.* (2002) on hypocotyl elongation and cell wall loosening. There still plenty of unknown aspects regarding Hg's genotoxicity, namely, the mechanistic, target, and extent of its effects in plants. Plants play a major role in trophy chains and economy hence, there is need to find process about uptake mechanism of Hg and protect our valuable ecosystem.

5. CONCLUSION

As it has been explored throughout this paper, Hg is a critical pollutant causing toxic effect both at morphological and physiological level. Hg can be easily spread through many ecosystems causing several toxic effects in many biological processes. The present investigation is of fundamental importance as it will reveal potential aspects of Hg toxicity on nitrase, an enzyme of nitrogen assimilation pathway. Therefore the present study will definitely help in assessing/evaluating the effect of Hg on overall productivity of important crops and detoxification of Hg by phytochelatin synthesis in Hg enriched soil.

Author Contributions

The tasks and responsibilities of the work assignment were equally shared by both the authors.

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