Co-exposure Effects of Selenium and Mercury on *Phaseolus vulgaris*

Excised Leaves Segment by Enhancing the NR, Anti-oxidative Enzyme Activity and Detoxification Mechanisms

Shilpa Shrivastava*, Archana Shrivastava and Jot Sharma

*Department of Biotechnology, College of Life Sciences, CHRI Campus, Gwalior, Madhya Pradesh, India
1Department of Microbiology, College of Life Sciences, CHRI Campus, Gwalior, Madhya Pradesh, India
2Department of Biotechnology, Jiwaji University, Gwalior, Madhya Pradesh, India

Abstract

Mercury is known to disrupt the biological function in plants by inhibiting their growth and developmental process, while selenium (Se) is an essential micronutrient within the appropriate amount. This paper is aimed to study co-application and interactive effects of selenium (Se) and mercury (Hg) on the Nitrate Reductase NR (such as in-vivo and endogenous) and the antioxidant system through a pot experiment and clarify the possible mechanism how Se alleviates the toxicity of Hg. The observations indicate that when selenium applied after mercury exposure the enzyme activity enhanced hence Se may reduce the toxic level of Hg in *phaseolus vulgaris*.

Keywords: Selenium; Mercury; Nitrate reductase; Endogenous; *Phaseolus vulgaris*

Introduction

Agricultural useful soils in many parts of India and the world are slightly contaminated by presence of heavy metal toxicity. Few as Cd, Cu, Zn, Ni, Co, Cr, Pb, and As. This because long-term use of phosphatic fertilizers, sewage sludge application, dust from smelters, industrial waste and bad watering practices in agricultural lands [1-3]. 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 [4].

Selenium (Se) is an essential micronutrient and has important benefits for animal and human nutrition, but in trace amount after that limit it causes toxicity in human and plants [5,6]. The presumed protective effect of Se against cadmium and mercury toxicity is through the diversion in their binding from low molecular weight proteins to higher molecular weight ones [7]. The Se distribution pattern was found to be unaffected by the presence of Hg, but the amount of Se assimilated was found to be higher in plants co-exposed to Hg [8].

However, until now the study of interaction of selenium and mercury in *phaseolus vulgaris* plants have not yet been reported. In this study, pot culture method under lab condition was used to study the interaction between (rajmah) selenium and mercury, which was of practical significance.

Materials and Method

Plant growth and metal treatment

Seeds of *phaseolus vulgaris* were rinsed in running water for 2 min. Seeds were surface sterilized with 0.1% HgCl₂ for 30 sec. and then washed with three times autoclaved double distill sterilized water. Sterilized seeds were sown on plastic pot containing 1 kg acid washed sand and 20 seeds per pot. Pots were placed in continuous light 30 w/m² intensity supplied by fluorescent tubes at 26 ± 2°C for 7-8 days. Half strength Hoagland solution without nitrogen was used for watering. Mercury Chloride (HgCl₂) and Sodium Selenite (Na₂SeO₃) 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.

Metal treatment: Treatment with metal at two levels in order to correlate uptake, accumulation and comparison of the same with the following treatment schedules.

a) Seeds were treated with Hg metals for 2 hrs and 4 hrs followed by thorough wash and subsequently planted on acid washed sand contained in plastic pots.

b) Different concentration of metal treated acid washed sand for 24 hrs contained in plastic pots.

Leaves floating: For Hg treated soil (for 24 hrs) and seed treated (two and four hour) in continuous light leaf were floated on 1/4th strength Hoagland solution. The young two cotyledons leaves were co treated with selenium (c (without treatment, 0.001, 0.01, 0.1, 1 mM) different concentration to see effect on different enzymes and correlate with detoxification mechanism. Incubation in continuous light inside “Indosan growth chamber” BOD. The leaf is then used in estimation of nitrate reductase *in vivo* and endogenous nitrate pool, peroxidase and catalase assay.

Nitrate reductase assay: The activity of nitrate reductase was estimated by *in vivo* nr by Srivastava [9] method with slight modification. Briefly 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 KNO₃, and 25% iso-propanol 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 colour development by the formation of diazo compound with sulfanilamide and nitrate coupled with NED to give a red
Excised Leaves

91.06 ± 6.48**
15.59 ± 1.33
0.21 ± 0.01
0.76 ± 0.25
31.68 ± 6.8
11.19 ± 4.38**
42.87 ± 3.76
16.95 ± 0.52**
1.44 ± 0.15**
0.84 ± 0.36**
28.57 ± 0.22**
35.71 ± 0.18**
7.99 ± 0.79**
12.03 ± 2.17
76.89 ± 3.68**
1.23 ± 0.25
1.52 ± 0.18
2.88 ± 0.15**
2
32.15 ± 1.75**
80.01 ± 7.63**
24.55 ± 4.18**
0.99 ± 0.06**
1.03 ± 0.18**
90.36 ± 6.38**
1.44 ± 0.15**
2.88 ± 0.15**
45.13 ± 4.49
31.29 ± 0.88**
24.07 ± 0.51
2.34 ± 0.27**
10.86 ± 0.71**
25.44 ± 0.68
27.92 ± 0.48**
35.27 ± 0.18**
11.46 ± 14.67**
treatment whereas in four hour activity shows constant result in all concentration (Table 3).

Hg inhibited endo nr activity in seed treated two hour at 0.1 mM concentration whereas in four hour 0.001 mM concentration significantly (Tables 2 and 3). When Se supplied with mercury, enhanced activity in 0.001 then 0.1mM concentration was observed during two hour seed treatment in comparison to this four hour seed treatment shows maximum activity in 0.001 mM than 0.1 and 0.01 mM concentration (Tables 2 and 3).

Antioxidant enzymes: Peroxidase activity when Hg treatment supplied to seed with two hour only in 1mM conc shows significantly increase in activity whereas in four hour 71 times more fold inhibition in 0.1mM followed by 0.001mM conc. or least inhibition in 0.01 mM conc. When Hg was co exposure supplied with selenium, peroxidase activity at 0.001 mM and 0.1mM shows more inhibition and 100 times more enhanced activity than control in both two hour and four hour (Tables 2 and 3).

During catalase activity, when only Hg treatment was given during seed treatment with two hour only in 1mM conc shows significantly decrease in activity whereas in four hour 93 times more fold inhibition in 0.1mM followed by 0.001mM conc. or least inhibition in 0.01 mM conc. When Hg was co exposure with Se mercury treatment supplied the activity enhanced and shows more detoxification. During two hour 0.1 mM shows more inhibition than 0.01mM, 0.001M and 1 mM with compare to control. During four hour, 0.01 mM and 0.1mM shows more inhibition (Tables 2 and 3).

Discussion and Conclusion

Obtained from the present study showed reduced enzyme activity under Hg stress which might be associated with inhibited contribution of essential component required for the particular enzyme activity of plant. Similar research also done earlier for identify mercury toxicity on plants or ecosystem. The growth pattern of phaseolus seedlings in the presence of Hg was different from pea and spear mint [15], ryegrass [16,17], tomato [18], Sesbania grandiflora [19], Mentha arvensis [20].

The activities of anti-oxidative enzymes in the seedlings of Phaseolus aureus found that effects of Hg and cadmium (Cd) had little primary damaging effect on membranes [21]. Antioxidant effect of HgCl₂ was in selected plant Cittoria ternatae L. It increases H₂O₂ content and the antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) were observed in HgCl₂ treated plants when compared with control [22].

Hg causes phyto toxicity and oxidative stress in wheat (Triticum aestivum L.) plants [23]. Plants treated with higher concentration of Hg were subjected to comparatively greater oxidative damage and demonstrated that the antioxidative components were not able to remove the stress due to higher concentration of Hg and thus might affect the productivity in plants. Hg also showed inhibition property towards physiological parameters such as chlorophyll, protein, nitrate, and endogenous pool. Higher concentrations of HgCl₂ were found to be more toxic [24].

The selenium content and species of both plant and animal foodstuffs depend on environmental conditions, in particular, the quantity and species of selenium to which the animal/plant is exposed. Selenomethionine is predominant in cereals, and selenium concentrations vary from 0.01 to 0.55 μg/g fresh weight [25], whereas in other plant foods the content is generally lower. Se existence supports the expression of the selenoprotein, which have important antioxidant and detoxification function [26]. These forms of selenium can combine with Hg⁺ to form insoluble Hg-Se complex in rice root surrounding environment or root surface [27].

Co-exposure of Se with Hg enhanced NR enzyme activity and improved antioxidant mechanisms seedlings which could be attributed to their synergistic effect and the role of Se to regulate the plant growth and detoxifying mercury toxicity.

The interaction between mercury (Hg (II)) with selenium Se (VI) and Se (IV) in Tomato (Lycopercium esculentum) experimented in Sand and soil culture resulted in Decreases Hg uptake [28]. Another study shows that Hg (II) and Se (VI) and Se (IV) in Radish plants (Raphanus sativus) performed in Pot culture decreases Hg uptake and possibly forms Hg-Se insoluble complex in soil [29]. Mercury Hg (II) Se (IV) in Glicyne max (soybean) experimented with Soil and a 50:50 mix of soil and ProMix resulted forms a high molecular weight entity containing Se and Hg in plants [8].

Se may play an important role in limiting the bioaccessibility, absorption, and translocation/bioaccumulation of mercury in the aerial rice plant, which may be related to the formation of an Hg-Se insoluble complex in the rhizospheres and/or roots [30].

Se antagonistic study with other heavy metal also studied such as arsenic, cadmium etc. Arsenic interaction with Se in Hydroponic culture and found that decreases As uptake and lipid peroxidation; enhances levels of antioxidants, chlorophyll, MTs, thiols and GST; increases cellular viability, and rebuilds membranes. Enhances As concentration, Se induces more production of thiols and GSH to counterbalance the negative effects of increased As and inhibits lipid peroxidation in Pteris vittata L. [31,32]. In Garlic (Allium sativum) found decreases Cd uptake and stimulates growth of root and seedlings. Another study in Rape (Brassica napus L., Polish genotype cv. Bojan) and wheat (Triticum aestivum, Polish genotype cv. Kamila and a Finnish genotype cv. Manu) results shows Promotes growth, reduces lipid peroxidation, enhances membrane stability, and counterbalances the Cd-induced changes in nutrients [33,34] (Figures 1-3).
The robust data presented in the above work shows that co-exposure of Se is more effective in the alleviation of Hg stress. Improved Hg tolerance in the presence of Se involves physiological, and biochemical interaction in a synergistic or additive way. Therefore, the present study gives a new strategy to minimize the Hg toxicity in crop plants, especially in rajmah (Phaseolus vulgaris) using beneficial application of Se as protective mechanism against mercury toxicity.

Acknowledgment

Sincerely obliged to Dr. Archana Shrivastava and Dr. Jot Sharma for their encouragement and providing necessary administrative and research facility.

References

1. Bell FG, Bullock SET, Haltibich TFJ, Lindsay P (2001) Environmental impacts associated with an abandoned mine in the Witbank Coalfield, South Africa. International Journal of Coal Geology 45: 195-216.
2. Schwartz C, Gerard E, Perronnet K, Morel JL (2001) Measurement of in situ phytoextraction of zinc by spontaneous metallophytes growing on a former smelter site. Science of the Total Environment 279: 215-221.
3. Passariello B, Giulano V, Quaresima S, Barbaro M, Caroli S, et al. (2002) Evaluation of the environmental contamination at an abandoned mining site. Microchemical Journal 73: 245-250.
4. Sahni SK (2011) Hazardous metals and minerals pollution in India. A Position Paper, August 2011. Indian National Science Academy, New Delhi.
5. Ellis DR, Salt DE (2003) Plants, selenium and human health. Curr Opin Plant Biol 6: 273-279.

Figure 1: Showing co-exposure of selenium with mercury during sand treatment. On x axis concentration of metal treatment in mM and on y axis inhibition of different enzymes.

Figure 2: Showing co-exposure of selenium with mercury during two hour seed treatment. On x axis concentration of metal treatment in mM and on y axis inhibition of different enzymes.

Figure 3: Showing co-exposure of selenium with mercury during four hour seed treatment. On x axis concentration of metal treatment in mM and on y axis inhibition of different enzymes.
31. Malik JA, Goel S, Kaur N, Sharma S, Singh I, et al. (2012) Selenium antagonises the toxic effects of arsenic on mungbean (Phaseolus aureus Roxb.) plants by restricting its uptake and enhancing the antioxidative and detoxification mechanisms. Environmental and Experimental Botany 77: 242-248.

32. Srivastava M, Ma LQ, Rathinasabapathi B, Srivastava P (2009) Effects of selenium on arsenic uptake in arsenic hyperaccumulator Pteris vittata L. Bioresour Technol 100: 1115-1121.

33. Zembala M, Filek M, Walas S, Mrowiec H, Korna’s A, et al. (2010) Effect of selenium on macro and microelement distribution and physiological parameters of rape and wheat seedlings exposed to cadmium stress. Plant and Soil 329: 457-468.

34. Xinbin Z, Wenhua W, Shuhui Y, Yongxiang Z (2013) Interactive Effects of Selenium and Mercury on Their Uptake by Rice Seedlings. Research Journal of Applied Sciences, Engineering and Technology 5: 4733-4739.