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Original Research Paper

Growth and Physiological Responses of the Seedling of *Raphanus sativus* Following Exposure of Seed to Mercury

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ABSTRACT

Heavy metal contamination has been incriminated to affect the physiological processes in plants and induces phytotoxicity at higher concentrations. The present investigation was carried out to assess the effect of 10-fold increase in concentrations of mercury on seed germination, seedling growth and biochemical parameters in axis and cotyledon of *Raphanus sativus*. The seeds sterilized by 80% ethanol were pre-soaked in 10^{-5}, 10^{-3} and 10^{-2} M concentrations of $HgCl_2$ for 24 hr. The control seeds were soaked in distilled water. These seeds were plated in moist filter paper and were kept in dark chamber at 25±2°C for 96 hours. Germination of seeds was recorded at 24 hr interval up to 96hr. The root and the shoot length were recorded in 48, 72, 96 and 120hr of radical emergence. *R. sativus* seeds showed 85, 77, 51 and 1% germination in control, 10^{-5}, 10^{-3}, 10^{-2} M mercury treated seeds, respectively after 24hr of plating. There was a significant inhibition of germination percent at 10^{-3} and 10^{-2} M Hg concentration. Maximum seedling growth was observed in control and minimum in 10^{-3} Hg during 120hr of seedling growth. Hg (10^{-5} and 10^{-3} M) inhibited hypocotyls growth by 10 to 40%, and radical growth by 15 to 50% as compared to control. The starch, sugar and amylase activity in the axis significantly ($P<0.05$) reduced with exposure of seed to 10^{-3} M concentration of mercury. It was concluded that the higher concentration of mercury significantly affects the seed germination and seedling growth.

INTRODUCTION

Emission of toxic heavy metals has risen tremendously during the past 200 years due to several anthropogenic activities. Uptake and accumulation by crops represents the main entry pathway for potentially health-threatening toxic metals into human and animal food (Clemens 2006). Mercury is one of the toxic metals that enters into the living system, and tends to accumulate in the nearby vegetation and interferes various physiological processes and induces phytotoxicity at higher concentrations. The bioavailable mercury concentration has increased tremendously with unplanned industrial developments, urbanization, improper and overuse of fungicides, pesticides and fertilizers containing this hazardous pollutant. A recent global assessment from the UN Environment Programme has documented that the deposition rate of Hg has grown by 1.5-3 during the past century because of increased anthropogenic emissions from industry and agriculture and from medical and domestic sources (UNEP 2002). About 75% of the mercury emissions are attributed to the combustion of fossil fuels, particularly coal combustion in China, India, and South and North Korea (Pacyna et al. 2003). Atmospheric Hg from combustion of coal can be transported over a long distance and deposited far from its origin (Pacyna & Pacyna 2002), thus elevating the mercury availability, its uptake by plants and consequent effects on physiological processes.

Raphanus sativus (radish) is a rampantly cultivated plant in various parts of India, and is used as a common vegetable. It is an edible root vegetable of the Brassicaceae family that was domesticated in Europe in pre-Roman times. Radishes have numerous varieties, varying in size, colour and duration of required cultivation time, and are grown and consumed throughout the world. The yield of the crop is determined by the seed germination patterns including seedling growth. The content of heavy metals and the associated phytotoxicity greatly influences the seedling growth in plants (Di Salvatore et al. 2008). The present investigation is carried out to study the effect of mercury exposure of varying concentrations on seed germination percent, seeding growth including shoot and length characteristics, and biochemical characteristics in axis and cotyledon of *Raphanus sativus*.

MATERIAL AND METHODS

Seeds: The seeds of *Raphanus sativus* were collected from Seed Sales Centre of Horticulture Department of Sambalpur. Those were surface sterilized with 80% ethanol for one minute followed by washing in distilled water.

Seed treatment: Sterilized seeds were pre-soaked in different concentrations of $HgCl_2$ (10^{-5}, 10^{-3}, 10^{-2}M) for 24 hr. The control seeds were soaked in distilled water. These seeds were

plated in moist filter paper and kept in dark chamber at 25±2°C for 96 hours. Three replicates with 25 seeds in each plate were taken for each treatment.

Germination and seedling growth: Germination of seeds was recorded at 24 hr interval up to 96hr. Protrusion of radicals was taken as criterion of germination and it was expressed in percentage. Seeds with equal radical length were chosen and plated in earthen pot filled with acid washed sand (Agarwal & Sharma 1976) and kept for 120hr for growth characteristics study. Each pot contained 25 seedlings. The root and the shoot length was recorded in 48, 72, 96 and 120hr of radical emergence. On every occasion, 10 saplings were taken for recording the observation.

Biochemical analysis: Biochemical analysis for starch, sugar and amylase activity was carried out at 24 hr from 48 hr to 120 hr in axis and cotyledon separately in the germinated seedlings in controls and mercury treated seeds with exposure to 10^{-5}M and 10^{-3}M Hg solution. The cotyledon and axis sampled at different hours were homogenized with 80% ethanol and the supernatant after centrifugation for 15 min were used for estimation of sugar content using anthrone reagent (Yemm & Willis 1954). The residue remaining after ethanolic extraction was used for estimation of starch (Mc Creedy et al. 1950). The samples of axis and cotyledon were ground separately with 5 mL of acetone buffer at pH 6 with a pinch of $CaCl_2$ and amylase activity was measured in the supernatant.

Statistical analysis: The data were analysed statistically to calculate mean ± S.E. and one way analysis of variance was performed to find out the significant difference between the control and treated seeds at particular observation period.

RESULTS AND DISCUSSION

The effect of mercury pretreatment of seeds on germination percentage of *R. sativus* at different hours is given in Table 1. *Raphanus sativus* seeds showed 85, 77, 51 and 1% germination in control, 10^{-5}, 10^{-3}, 10^{-2}M Hg treated seeds, respectively after 24hr of plating. In control and 10^{-5}M Hg treated seeds, germination continued up to 48hr whereas germination took 72 hr in cases of seeds treated with 10^{-3} and 10^{-2} Hg. There was a clear-cut inhibition of germination percentage at 10^{-3} and 10^{-2}M Hg concentration.

Table 2 shows root and shoot length of *R. sativus* seedling, and Table 3 the fresh and dry weight at 24hr interval till 120hrs after treatment of seeds with mercury. Increasing trend in elongation of shoot and root length was marked from 48 to 120hr. Both the root and shoot length decreased with increasing concentrations of Hg. Maximum seedling growth was observed in control and minimum in 10^{-3} Hg during 120hr. Hg (10^{-5}, 10^{-3}M) inhibited hypocotyl growth about

Table 1: Germination percentage after treatment of *R. sativus* seeds with different concentrations of mercury.

Mercury concentration	Hour of observation				% Germination
(in Molar)	24	48	72	96	
Control	83	2	0	0	85
10^{-5}	71	5	1	0	77
10^{-3}	45	4	2	0	51
10^{-2}	0	0	1	0	1

10 to 40% and radical growth by 15 to 50%, respectively as compared to control. There was only 1% germination by the end of 72 hr and no further seedling germination was recorded after 72hr.

Figs. 1 and 2 show the starch, sugar and amylase activity in the axis and cotyledon of the *R. sativus* seedling in control and mercury exposed seed (10^{-5} and 10^{-3}M). The starch, sugar and amylase activity in the axis significantly ($P<0.05$) reduced with exposure of seed to 10^{-3}M concentration of mercury. However, there was increase in starch content in the cotyledon in the group treated with 10^{-5} and 10^{-3} M $HgCl_2$. However, sugar and amylase activity was higher in unexposed group and treatment with Hg reduced the sugar and amylase activity in the cotyledon.

Mercury contamination of the soil is arguably of the greatest environmental and public health concern (Lomonte et al. 2011) because of its extremely high toxicity in both its organic and inorganic compounds and its ability to bioaccumulate, thus increasing the risks to human and animal exposure even at trace levels (Kelly et al. 2006). India is one among the world's most active mercury industrial centres. Chloroalkali industries are still the major source of mercury release into atmosphere and surface water (Lenka et al. 1992). Other industries, which contribute to mercury pollution in India, are coal fired plants viz., thermal power plants, steel industries and cement plants. Excess of mercury in biosphere is a major environmental hazard (Eisler 2004). Mercury concentration in the agricultural land may increase with application of sludge, fertilizers, lime and manures. The most important sources of contaminating agricultural soil have been the use of organic mercurials as a seed-coat dressing to prevent fungal diseases in seeds (Patra & Sharma 2000). Mercury was introduced for treatment of seed from 1915, and was stopped from 1982 because of effects on seed germination and seedling growth. However, injury to the seed increases in direct proportion to increasing rates of application.

Elevated inorganic mercury (IHg) and methylated mercury (MeHg) concentrations have been reported in rice plants

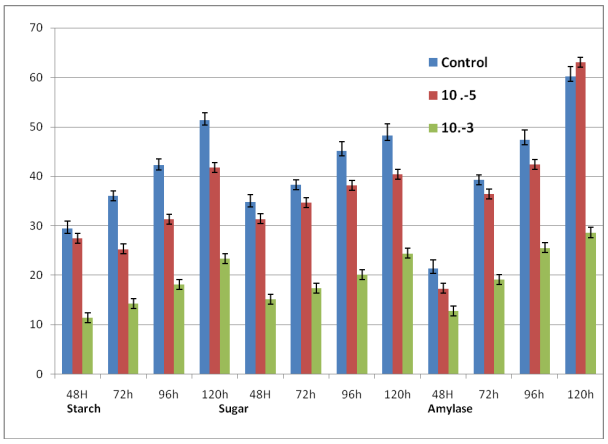

Fig. 1: Starch, sugar and amylase activity in the axis of *R. sativus* seedling following exposure of the seed to mercury at different hours.

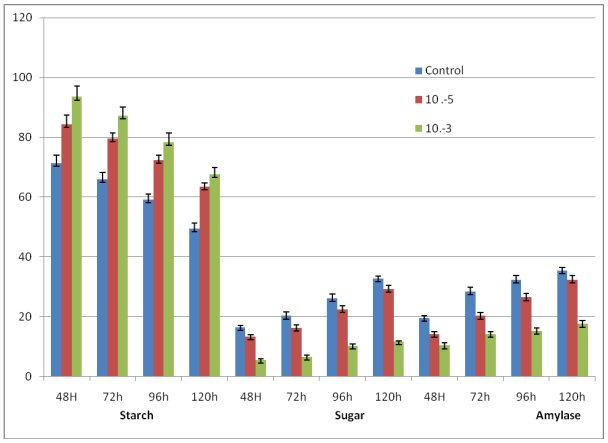

Fig. 2: Starch, sugar and amylase activity in the cotyledon of *R. sativus* seedling following exposure of the seed to mercury at different hours.

cultivated in Hg mining area, and the rice seeds have the highest ability to accumulate MeHg compared to the other tissues (Meng et al. 2010). Seed soaking treatment with different concentrations of mercuric chloride ranging from 0 to 3.0 mM has been conducted to study the phytotoxic effect of mercuric chloride on seed germination and seedling growth of maize var. Jaunpuri (Bose et al. 2008). In the present experiment, sterilized seeds of *R. sativus* were presoaked in different concentrations (10^{-5}, 10^{-3}, 10^{-2}M) of $HgCl_2$ for 24 hr.

Seed germination and seedling growth are the most important features that directly or indirectly determine the yield of a particular crop (Gelmond 1978). The present finding of germination percentage may be attributed to absorption of Hg present in the imbibition medium along with water during the process of germination (Lootang 1995). Mercury has affinity for SH group of protein (Jerome & Ferguson 1972). It might be possible that Hg inhibits the activity of hydrolytic enzyme required at the time of germination inhibiting the germination in *R. sativus*. Mercury induced inhibition of germination might also be attributed to non-availability of sugar at all the concentrations taken for this study and also due to injury of embryos caused by Hg (Mishra & Choudhary 1997). In the present finding, the highest concentration of mercury (10^{-2}M $HgCl_2$) showed only 1% germination by the end of the study, therefore, further biochemical analysis could not be carried out in the seedling with exposure of seeds to the highest concentration of the mercury. Singh et al. (2007) studied the response of wheat seed germination and seedling growth under copper stress, and physiological and protein profiles alterations have been reported in rice seedling exposed to acute cadmium toxicity (Ahsam et al. 2007). But, Agarwal et al. (1961) recorded enhanced germination of *Hordeum vulgare* when seeds were treated with mercury. However, the retardation of germination in the present investigation with exposure to higher concentrations of mercury in *R. Sativus* substantiates the earlier findings in *Oryza sativa* cultivar (Mukherjee & Maitra 1977), and *Zea mays* (Kalimuthus & Sivasubramanian 1990).

Exposure dependent poor seedling growth and biochemical parameters with increasing concentration of Hg might be attributed to restricted availability of potentially mobile matter through suppression of hydrolytic enzymes leading to decline in dry matter content in cotyledon and poor seedling growth (Sharma 1985). Inhibition of seedling growth at different concentrations of Hg can be compared with germination performance in *Glycine max* (Dubey & Dwivedi 1987), *Vigna mungo* (Nandi & Bera 1995). The reduction in root length was more than shoot length as recorded in the present investigation and reduced starch and sugar content in axis and sugar only in cotyledons might be due to direct contact of root to the solution containing higher concentration of mercury. The possible mechanisms of mercury toxicity are changes in the permeability of the cell membrane, reactions of sulphydryl (-SH) groups with cations, affinity for reacting with phosphate groups and active groups of ADP or ATP, and replacement of essential ions, mainly major cations (Patra & Sharma 2000). The tissue specific changes to different concentrations of toxic heavy metals are also on record (Sharma 1985). It is concluded that the higher concentration of mercury inhibits germination performance, seedling growth and alters the biochemical composition of the axis and cotyledon of *R. sativus*.

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Table 2: Root and shoot length of *R. sativus* seedlings at different hours after treatment of seeds with different concentrations of mercury.

Mercury concentration	Root length in cm at different hrs				Shoot length in cm at different hrs			
(in Molar)	48	72	96	120	48	72	96	120
Control	2.82 ± 0.52	4.13 ± 0.98	5.92 ± 0.56	7.31 ± 0.67	1.43 ± 0.86	2.29 ± 0.92	3.62 ± 0.75	4.30 ± 0.81
10^{-5}	2.43 ± 0.50	3.52 ± 0.86	4.95 ± 0.76	6.05 ± 0.35	1.27 ± 0.76	1.93 ± 0.83	3.16 ± 0.73	3.80 ± 0.68
10^{-3}	1.52 ± 0.32	2.29 ± 1.10	3.24 ± 0.37	4.10 ± 0.96	0.58 ± 0.52	1.36 ± 0.54	2.19 ± 0.45	2.00 ± 0.50

Table 3: Effect of mercury pretreatment of seeds on fresh and dry weight of *R. sativus* seedlings.

Mercury concentration	Fresh weight of seedlings at different hours				Dry weight of seedling at different hours			
(in Molar)	48	72	96	120	48	72	96	120
Control	49.31± 6.5	72.52 ± 6.9	90.84 ± 7.4	105.63 ± 7.8	3.35 ± 0.51	4.89 ± 0.71	6.62 ± 0.68	7.53 ± 0.53
10^{-5}	62.22 ± 5.5	79.28 ± 7.8	88.54 ± 9.6	99.56 ± 8.5	3.17 ± 0.43	4.56 ± 0.98	5.98 ± 0.81	7.32 ± 0.43
10^{-3}	27.44 ± 6.8*	43.37 ± 7.2*	51.25 ± 9.2*	56.76 ± 8.8*	1.75 ± 0.5*	3.54 ± 0.5*	4.59 ± 0.7*	4.92 ± 0.41*

*P<0.05 from control group at particular period of observation.

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