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Effect of mercuric chloride on seed germination and seed respiration of *Vigna radiata* (L.) Wilczek and *Vigna mungo* (L.) Hepper

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ABSTRACT

Mercury is a typical toxic trace metal and its bioaccumulation in plants and subsequent entry into the food chain results in long-term health hazards in human beings. During the present investigation, the effect of mercuric chloride on seed germination, radicle length, hypocotyl length and seed respiration of two commonly consumed leguminous crops viz. *Vigna radiata* (mungbean) and *Vigna mungo* (blackgram) were studied. Considerable reduction in the percentage of seed germination, radicle length, hypocotyl length and respiratory O_2 consumption in germinating seeds was observed with the increase of concentration of mercuric chloride. The effect of mercury was found to be dependent on dose and duration of exposure besides genetic factors.

1. Introduction

In recent years, there has been an increasing ecological and global public health concern associated with environmental contamination by heavy metals. Human exposure has also risen dramatically as a result of an exponential increase of their use in several industrial, agricultural, domestic and technological applications. The multiple industrial, domestic, agricultural, medical and technological applications of heavy metals have led to their wide distribution in the environment; raising concerns over their potential effects on human health and the environment (Bradl, 2002).

Their toxicity depends on several factors including the dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals. Because of their high degree of toxicity, arsenic, cadmium, chromium, lead, and mercury rank among the priority metals that are of public health significance. These metallic elements are considered systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure (Chang *et al*., 1996). In biological systems, heavy metals have been reported to affect cellular organelles and components such as cell membrane, mitochondrial, lysosome, endoplasmic reticulum, nuclei, and some enzymes involved in metabolism, detoxification, and damage repair (Wang & Shi, 2001).

Mercury is a widespread environmental toxicant and pollutant which induces severe alterations in the body tissues and causes a wide range of adverse health effects (Sarkar, 2005). Both humans and animals are exposed to various chemical forms of mercury in the environment. Because mercury is ubiquitous in the environment, humans, plants and animals are all unable to avoid exposure to some form of mercury (Holmes *et al*., 2009). Bioaccumulation of mercury in plants and its entry into the food chain resulting in long term health hazard is of major concern.

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Generally, legumes are richer in proteins than cereal grains. Of these, green gram (*Vigna radiata*) and black gram (*Vigna mungo)* are two important tropical grain legumes, which provide humans with the significant amount of dietary proteins. In view of their wide-spread consumption as food items, the effect of the heavy metal *i. e*. mercury on seed germination, radicle length, hypocotyl length and seed respiration in *Vigna radiata and Vigna mungo* were studied.

2. Materials and methods

2.1. The test chemical

Mercuric chloride ($HgCl_2$) was taken as the test chemical to conduct the experiments to evaluate its toxicity on the plant species. The molecular weight of the chemical is 271 g/mole.

2.2. Preparation of the stock solution

271 mg of $HgCl_2$ was dissolved in 100ml sterilized distilled water to prepare 10mM stock solution. The stock solution was kept in a refrigerator for its subsequent use in the experiment. The required experimental concentration of the metal was achieved by diluting the stock solutions with distilled water. The concentrations of $HgCl_2$ for the experiment ranged from 0 – 5mM.

2.3. The test plants

Two leguminous species such as *Vigna mungo* (blackgram) and *Vigna radiata* (Green gram or Mung bean) were selected for the toxicity study. The mature seeds were collected from Centre for Pulses Research (OUAT), Bhubaneswar.

2.4. Experimental details

One hundred and fifty seeds of each of *V. radiata* and *V. mungo* were soaked in tap water overnight. Of the soaked seeds, 20 viable seeds of each species were selected and placed over pre-moistened cotton in two sets of petridishes. Three petridishes were taken as replicates of each test concentration and the control was grown in cotton soaked with tap water. Thirty milliliters of $HgCl_2$ solution of concentration range i. e. 100µM, 200µM, 500µM, 700µM and 1000µM were added to each petriplate and two sets were prepared. The petriplates were kept under continuous light intensity of 70µE/m^2S provided from cool white fluorescent tubes, relative humidity of 65% and constant day and night temperature of 29°±2°C for observation of rate of germination, rate of respiration and measurement of hypocotyl and radicle length.

The number of germinated seeds in each petridish was counted at the interval of 18h, 24h, 42h and 48h. After 48h, the hypocotyl and the radicle were separated in each seedling and their lengths were measured. The length of hypocotyl and radicle of all the seedlings in each petridish were measured and their average was determined.

Respiration of germinating seeds was measured after 48h using an Infra Red Gas Analyzer (IRGA) (PP Systems, UK). The measurement was done under ambient light, CO_2 and temperature. The measurement of respiration was done under the respiration measurement mode of the instrument using a soil respiration chamber (SRC). Prior to the measurement, the respiration was flushed with air for 15 seconds after which it was placed above the petriplate containing germinating seeds. The petriplates were kept on a rubber pad to ensure that the cuvette (SRC) is placed airtight on the petriplate. The measurement was recorded for 1 min and for each samples three readings were taken at an interval of 5 minutes between two consecutive readings.

3. Results

In the present study, mercuric chloride ($HgCl_2$) was selected as the contaminant and the phytotoxic effect was studied on seedlings of *Vigna radiata* and *Vigna mungo.*

3.1. Effect on seed germination

The rate of germination of seeds of *Vigna radiata* and *Vigna mungo* with treatment of different concentrations of $HgCl_2$ is presented in Table 1 and 2 respectively. Significant decrease in the rate of germination was observed with increasing concentration of $HgCl_2$. The percentage of seed germination increased constantly with prolongation of incubation period and decreased with increasing concentration of the applied $HgCl_2$. As compared to control, the rate of germination was invariably lower in all seeds treated with $HgCl_2$. However, at a concentration of 5000 µM, the rate of germination becomes nearly constant for *Vigna mungo* irrespective of the period of exposure. Similarly, for *Vigna radiata*, at 2000 µM concentration, the percentage of seed germination reached almost a constant value at 18, 244, 42 and 48 hrs of treatment. Thus from the result it could be derived that the seed germination process might have been influenced by the toxic effect of $HgCl_2$. The level of toxicity and time of exposure varied between *Vigna radiata* than *Vigna mungo* and the later was more tolerant to $HgCl_2$ toxicity.

3.2. Effect on radicle and hypocotyl length

The lengths of hypocotyls were found to be almost double the length of radicles in both the species of *Vigna* as can be seen in Table 3. In untreated condition, the radicles and hypocotyls of germinating seeds of *Vigna radiata* were

Table 1

Rate of germination (%) of *Vigna radiata* seeds treated with different concentrations of Hg^{2+}

Concentration (μM)	Period of treatment			
	18 h	24 h	42 h	48 h
0	100 ± 2.42	100 ± 2.86	100 ± 3.18	100 ± 1.49
100	96 ± 3.59	98 ± 2.86	100 ± 2.95	100 ± 3.18
500	87 ± 2.62	88 ± 2.58	91 ± 2.68	93 ± 2.59
1000	76 ± 2.85	79 ± 2.18	79 ± 2.83	79 ± 2.63
2000	68 ± 1.63	68 ± 2.85	68 ± 1.93	68 ± 2.63
5000	52 ± 0.82	52 ± 0.86	53 ± 0.18	53 ± 0.95

Values are the means ±SD from 3 replicates

Table 2

Percentage of germination of seeds of *Vigna mungo* treated with different concentrations of Hg^{2+}

Concentration(μM)	Period of treatment			
	18 h	24 h	42 h	48 h
0	56 ± 1.83	66 ± 2.19	87 ± 2.86	97 ± 2.56
100	73 ± 1.86	75 ± 2.15	87 ± 2.86	98 ± 3.11
500	56 ± 0.63	57 ± 1.59	64 ± 2.04	72 ± 2.85
1000	32 ± 0.32	40 ± 1.28	48 ± 0.59	59 ± 1.38
2000	20 ± 0.25	29 ± 1.04	29 ± 0.85	32 ± 1.06
5000	20 ± 0.18	20 ± 1.01	20 ± 0.85	20 ± 0.63

Values are the means ±SD from 3 replicates

longer compared to that of *Vigna mungo*. The ratio of radicle length and hypocotyl length remained 1:2 approximately till the concentration reached 500 mM for *Vigna mungo*. Beyond this concentration, there was no noticeable growth of hypocotyls. In *Vigna radiata*, however, the length of hypocotyl and radicle became constant at 500 mM and at higher concentrations the growth of hypocotyls was negligible.

The results of the above experiment indicate that $HgCl_2$ has significant toxic effect on germination of seeds of *Vigna*

Table 3

Variation in length of hypocotyls and radicles of germinated *V. radiata* and *V. mungo* seeds treated with different concentrations of Hg^{2+}

Concentration(μM)	*Vigna radiata*		*Vigna mungo*	
	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)
0	3.228 ± 0.013	6.442 ± 0.039	2.853 ± 0.018	5.289 ± 0.128
100	1.735 ± 0.032	3.685 ± 0.028	1.483 ± 0.063	3.049 ± 0.142
500	1.357 ± 0.042	1.328 ± 0.042	0.975 ± 0.042	1.216 ± 0.056
1000	0.813 ± 0.024	0	0.426 ± 0.017	0
2000	0.852 ± 0.026	0	0.421 ± 0.029	0
5000	0.438 ± 0.014	0	0.429 ± 0.013	0

Values are the means ±SD from 3 replicates

mungo and *Vigna radiata* and growth of hypocotyls was inhibited to considerable extent. The radicle length remained constant after 1000 mM concentration in *Vigna mungo,* while in *Vigna radiata* there was a gradual shortening of radicle length with increasing concentration of $HgCl_2$. This leads to the conclusion that the germination process was triggered before the accumulation of significant level of the toxic metal in the seeds leading to a higher rate of germination of seeds of both the test species. Subsequently, due to metal uptake and accumulation, the germinated seeds did not grow further and there was wilting and degeneration of the emerged radicles.

3.3. Effect on seed respiration

In each treatment, $HgCl_2$ caused significant decrease in the rate of respiratory O_2 consumption (Table 4) by the germinated seeds and emerged seedlings. The inhibitory effect increased with increasing concentrations of the metal solution for both the test species. At 5000 µM concentration, there was no measurable respiratory consumption of O_2. This indicates that the metal completely inhibited the activity of cells at 5000 µM concentration. The respiratory O_2 consumption at 1000 µM concentration of $HgCl_2$ was about 30% of that observed in control.

Table 4

Respiratory O_2 consumption of germinated seeds of *V. radiata* and *V. mungo* after 2 days of treatment with different concentrations of Hg^{2+}

Concentration (µM)	*Vigna radiata*	*Vigna mungo*
0	1.365 ± 0.005	1.025 ± 0.017
100	1.085 ± 0.038	0.956 ± 0.028
500	0.916 ± 0.049	0.427 ± 0.013
1000	0.439 ± 0.028	0.315 ± 0.015
2000	0.214 ± 0.017	0.104 ± 0.008
5000	0	0

Values are the means ±SD from 3 replicates

4. Discussion

It was observed that rate of seed germination in *V. radiata* and *Vigna mungo* decreased with the increase in concentrations of $HgCl_2$, which suggests that the seed germination process is influenced adversely due to toxic effect of mercury. Similar trend was also noticed in length of radicles and hypocotyls of germinating seeds. Further, it was observed that the respiration consumption at 0.1ml is about 30% compared to control. Possibly, with increase of concentration of $HgCl_2$ the amount of oxygen liberated increased and consumption also increased directly affecting the seed respiration and this has possibly resulted in reduction of the rate of seed germination and growth of radicles and hypocotyls. Mor *et al*. (2002) and few other researchers observed that the Hg contaminated soil retards the rate of seed germination and elongation of hypocotyls. The toxic effects of heavy metals such as lead, manganese and arsenic etc. have been reported by several workers (Chakrabarty *et al*., 1989; Joardar *et al*., 1988; Bandyopadhyaya *et al*., 1997).

Several studies have demonstrated that plant roots accumulate Hg when they are exposed to Hg-contaminated soils (Lenka *et al*., 1992) and plant roots accumulate more amount of Hg than shoots.

The result obtained during the present study revealed that heavy metal like mercury get accumulated in seeds and has profound toxic effects on seed germination and seedling establishment. The accumulation of the metal in seeds and other plant parts from soil and subsequent consumption by human beings is likely to have adverse effect on health.

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