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Original Research Paper

Effect of Mercury Exposure on *Vigna unguiculata* (Cowpea) Seeds

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ABSTRACT

Vigna unguiculata (cowpea) seeds were grown under controlled environment in solution of mercury at different concentrations. The eco-toxicity of mercury on the seed germination, early seedling growth, metabolite level, enzyme activities and the uptake of heavy metal content in the seeds were tested. It may be concluded that the inhibition of germination, rate of germination and growth of seeds of *Vigna unguiculata* in response to mercury demonstrate that they are suppressed not only the protrusion of radical or delayed the germination but also retarded the growth. The seedling growth is more sensitive to heavy metal (Hg^{2+}) in comparison to seed germination. Mercury treatment is potentially toxic to *Vigna unguiculata* root than shoot. Absorption depends on the ability of the root and may have an influence on synthesis of protein, carbohydrate, activity of enzyme and phenolic content, some of which can be enhanced, while others get inhibited. The sensitivity of *Vigna unguiculata* seeds in terms of biochemical changes and enzyme activities was remarkably noticeable. The results suggest that biochemical changes and enzyme activity in *Vigna unguiculata* plants is promising indicator of heavy metal toxicity. *Vigna unguiculata* assay should be further explored so that its value can be evaluated when more data are available.

INTRODUCTION

Mercury, a silvery liquid metal at room temperature was first identified as a human toxin in 1866 when two laboratory technicians who worked with the metal died of dimethyl mercury poisoning after short exposure periods (Wantanabe & Hiroshi 1996). Mercury is a cumulative heavy metal poison which occurs in its elemental form, inorganically as salts, or organically as organomercury compounds; the three groups vary in effects due to differences in their absorption and metabolism, among other factors (Sweet et al. 2001). Mercury damages the central nervous system, endocrine system, kidney and other organs, and adversely affects the mouth, gums and teeth. Exposure over long periods of time or heavy exposure to mercury vapour can result in brain damage and ultimately death. Mercury and its compounds are particularly toxic to fetuses and infants. Women who have been exposed to mercury in pregnancy have sometimes given birth to children with serious birth defects. Mercury exposure in young children can have severe neurological consequences, preventing nerve sheaths from forming properly. Mercury inhibits the formation of myelin, the building block protein that forms these sheaths. Plants which are exposed to mercury accumulate the metal, and drastic decrease in growth is usually observed. Plants exposed to ionic mercury through the root exhibit reduced growth of shoots and roots. They also accumulate mercury in the root with slow movement to the shoot. Tree leaves can trap atmospheric mercury. It is thought that inorganic mercury may cause changes in root tip cell membrane integrity while methyl mercury may affect organelle metabolism processes that eventually interrupt cell membrane integrity. The exposure of

plants to mercurials may be by: 1. Direct administration as antifungal agents, i.e., mainly to crop plants, through seed treatment or foliar spray. The end points screened are seed germination, seedling growth, relative growth of root and shoot and in some cases studies of leaf area index, internode development and other anatomical characters. 2. Accidental exposures through soil, water and air pollution. The level of toxicity is tested usually under laboratory conditions using a wide range of concentrations and different periods of exposure. Additional parameters include biochemical assays and genetical studies (Patra & Sharma 2000).

Vigna unguiculata belongs to the family Papilionaceae. It is an annual plant and grow up to 10-20 ft in height. These plants mature within 80-100 days and vegetative in nature. The seeds of these plants undergo open seed pollination. Because of its early growth, seeds of *Vigna unguiculata* are often selected for the toxicity studies. Seed is a development stage which is highly protective against external stresses in the plant life cycle. This study was done to determine the effect of mercury on the seed germination; early seeding growth, metabolite level, enzyme activities and the uptake of heavy metal content in the seeds.

MATERIALS AND METHODS

Cowpea *(Vigna unguiculata)* seeds were obtained from seed technology division, Tamilnadu Agricultural University, Coimbatore and used for germination. The chloride salt formulation and mercury metal were from Qualigens chemicals. The selected concentrations of Hg (as $HgCl_2$) were between 0.05-2.0 mM, because of its high inhibitory effect. All preparations were made in doubly distilled water and comparison were made using distilled water only as controls.

The effect of each concentration of the metal was studied on 20 seeds of cowpea (*Vigna unguiculata).* Seeds were washed in distilled water and sterilized by immersion in a 0.1% mercury chloride solution for two minutes followed by five times washing in double distilled water and placed for incubation at 28°C for 24 hrs. Seed germination experiments were performed by taking distributed seeds in a Petri dish over the surface of Whatman No. 1 filter paper moistened with 10mL of heavy metal solution of mercury, whereas double distilled water was taken as control. Three replications for each concentration of Hg were taken for all the treatments. The covers of the Petri dishes were closed and kept at room temperature in the illuminated cabinet for 15 days. Germination percentage, root and shoot length (mm) and dry weight of root and shoot, were determined at 5, 10, 15 and 20 days. The number of germinated seeds was counted for every 24 hour for 3 days and the data were expressed as percentage of the total number of seeds used. The emergence of radicle from seeds was considered as germination. The root and shoot lengths of germinated seeds were measured only after 3 days incubation to the total experimental period. The preliminary studies showed that this duration is required to determine the inhibitory effects of Hg. Root and shoot samples were separated after the incubation of 15 days and washed in distilled water 3 times and dried at 85°C for 48 hours then dry weights were taken. Biochemical characterization included carbohydrate (anthrone reagent method), protein (Lowry's method), non-reducing sugars and reducing sugars (dinitro salicylic acid method), starch (anthrone method), phenol (Folin-Ciacalteau reagent method) and peroxidase (Guaiacol reagent method).

RESULTS

Seeds of *Vigna unguiculata* germinated under the heavy metal (Hg^{2+}) treatment, but the heavy metal treatment caused significant reduction in germination of seeds with increase in the concentration

(Table 1). A considerable decrease in dry weight of root and shoot was observed with increase in concentration of mercury than control from day 1 to day 15. The radicle and shoot length were significantly inhibited by mercury; the degree of inhibition varied depending on the concentration of heavy metal (Table 2). Elevated concentration of mercury resulted in greater accumulation of metal in root and shoot. Root accumulated higher concentration than shoot (Table 2). Mercury stress decreased amylase activity but increased peroxidase activity when compared with control (Table 3). High levels of mercury were found to increase the level of phenolic compounds than the control (Table 3).

The protein content at the imbibition stage was higher, whereas the treated sample showed less content than that of control (Table 3). Higher concentration of Hg caused reduction in carbohydrate as well as starch, and non-reducing and reducing sugars thereby reducing the accumulation of soluble sugar (Table 4).

DISCUSSION

Mercury produced a reduction in the germination of *Vigna unguiculata* seeds. Mercury even at low concentration (0.5 and 2 mM) is more toxic in reducing seed germination. This may due to ion toxicity associated with changes in cellular permeability, inhibition of protein activity and direct toxicity to the embryo and seedling (Dubey & Dwivedi 1987).

Lower reduction in dry weight of root and shoot was observed with 0.05mM of mercury. At 2mM of $HgCl_2$, root failed to germinate, which could be due to accumulation of mercury in the seedlings with increased concentration in soaked solution. A decrease in dry weight of shoot could be due to the inhibition of hydrolysis of reserve or synthesis of food and its translocation to the

Table 1: Effect of mercury on the germination percentage of cowpea seeds and root and shoot dry weight.

S.No.	Concentration (mM)	Germination (%) (5 days incubation)	Root and shoot dry weight (mg/seedling) (15 days incubation)	
			Root	Shoot
1	0.00	96.0	6.0	8.1
2	0.05	66.0	1.2	1.9
3	0.10	61.0	1.0	0.9
4	0.50	16.0	0.6	0.7
5	1.00	14.0	0.03	0.05
6	1.50	6.0	0.02	0.04
7	2.00	2.0	0.01	0.02

Table 2: Effect of mercury on root and shoot length and mercury cocentration in root and shoot.

S.No	Conc. (mM)	Root length (mm)			Shoot length (mm)			Hg_2Cl_2 conc. (µg/g)	
		5th day	10th day	15th day	5th day	10th day	15th day	Root	Shoot
1	0.00	15.00	20.00	25.00	20.00	49.00	56.00	0.00	0.00
2	0.05	4.70	9.80	14.50	14.50	30.00	41.00	24.4	1.06
3	0.10	2.50	3.70	6.80	7.80	18.00	23.00	38.6	1.90
4	0.50	0.40	0..90	4.30	5.20	11.00	15.00	85.5	2.40
5	1.00	0.30	0.60	1.00	2.00	6.00	9.00	116.4	4.10
6	1.50	0.20	0.35	0.50	0.90	2.00	5.00	124.0	15.40
7	2.00	0.05	0.06	0.10	0.40	0.50	1.00	131.5	20.20

Table 3: Effect of mercury on amylase, peroxidase, phenol and protein.

S.No.	Conc. (mM)	Amylase (Units/g of fresh weight)	Peroxidase (Units/g of fresh weight)	Phenol (g/l00g)			Protein (g/l00g)		
				1-D	7-D	15-D	1-D	7-D	15-D
1.	0.00	241.3	266.2	2.6	3.7	4.1	14.2	16.9	18.6
2.	0.05	243.6	272.5	3.5	3.8	5.2	14.3	16.7	17.3
3.	0.10	235.1	278.4	3.4	5.6	6.0	14.1	16.0	16.8
4.	0.50	212.4	269.6	4.6	5.4	6.1	14.2	15.8	16.3
5.	1.00	169.7	289.7	4.5	5.2	8.4	14.1	14.9	11.4
6.	1.50	142.5	318.4	6.3	6.3	8.6	13.9	14.1	12.7
7.	2.00	141.7	296.2	5.9	7.0	38.9	12.8	16.6	9.0

Table 4: Effect of mercury on carbohydrate, starch, and reducing and nonreducing sugars.

S.No	Conc. (mM)	Carbohydrate (g/l00g)			Reducing mg/g/dry wt			Non reducing mg/g/dry wt			Starch mg/g/dry wt		
		1-D	7-D	15-D	1-D	7-D	15-D	1-D	7-D	15-D	1-D	7-D	15-D
1	0.00	38.6	41.5	42.1	3.8	4.8	5.3	14.5	12.4	17.1	22.5	26.4	28.9
2	0.05	37.4	42.4	42.1	3.9	4.2	2.9	11.52	10.2	9.1	21.7	25.8	27.8
3	0.10	37.3	43.1	42.1	3.5	4.4	2.1	12.6	11.6	8.2	19.8	24.7	26.7
4	0.50	37.4	41.2	41.1	3.8	2.6	2.6	13.1	11.7	7.8	22.7	24.3	20.2
5	1.00	37.2	40.0	40.2	3.4	2.1	1.8	12.8	10.2	6.7	23.1	9.6	15.3
6	1.50	36.4	39.0	38.0	2.8	2.3	1.6	13.7	9.6	8.1	20.6	18.1	16.7
7	2.00	36.5	37.0	37.0	2.9	2.0	1.5	13.8	8.9	9.1	20.5	18.2	16.9

growing axis. Presoaking of seed in water that served as control increased dry weight and protein content compared to high concentration of mercury. This is due to the fact that presoaking of seed increased the potential of seedling to extract more moisture from the atmosphere due to change in lipophilic colloids (Chinoy et al. 1970).

Treatment at lower concentration of Hg (0.05-0.1 mM) affected radicle length adversely compared to shoot length, which was much higher at 2mM concentration. The radicle did not grow further and totally stopped at 2mM concentration. According to Iqbal & Naz (2005) seed germination and shoot length of *Thespesia populnea* showed significant reduction by mercury treatment. The availability of soil Hg to plants is low, and there is a tendency for Hg accumulation in the roots, indicating that the roots serve as a barrier to Hg uptake. Mercury concentration in above ground parts of plants appears to largely dependent on foliar uptake of HgO volatilised from the soil (Patra & Sharma 2000).

Localization of mercury was greater in root than shoot. The varied response to Hg concentration could be due to differences in absorption and accumulation in radicle. The rapid absorption by root and faster rate of detoxification in the root leads to more accumulation in the root (Shaukat et al. 1999). Metal uptake by grains seemed to be directly related to the concentration of heavy metals and was greater in the case of an individual metal added separately than in combination (Rana Athar & Masood Ahmad 2002).

The increased activity of peroxidase may be due to high salinity stress. The activities of enzyme peroxidase directly correlated with treatment levels. The increase in phenolic compounds can inhibit germination and growth. Phenolic acid excretes a marked effect on membrane permeability and mem-

brane electrical potential (Glass & Dunlop 1974). Oxidative damage is also exacerbated by environmental stresses as diverse as drought, high salinity, temperature, extremes, excess light, UV-B radiation, toxic metals and air pollutants. This has spurred attempts to develop transgenic plants overexpressing antioxidant enzymes in the hope of causing a generalized increase in stress resistance (Jain et al. 2005). Transition metals cause oxidative injury in plant tissues, but a literature survey did not provide evidence that this stress could be alleviated by increased levels of antioxidative systems. The reason may be that transition metals initiate hydroxyl radical production, which can not be controlled by antioxidants. Exposure of plants to non-redox reactive metals also resulted in oxidative stress as indicated by lipid peroxidation, H_2O_2 accumulation, and an oxidative burst (Schutzendubel & Polle 2002).

The increase in protein content during germination is well known, as it is increased with hydration, as the metabolism gets activated and the structural and functional proteins are synthesized (Osbome 1924). Retardation of the anabolic system results in lower content of the proteins in treated samples compared to control showing effectivity of mercury in inhibiting the synthesis of macromolecule during initial stage with no significant change in the content during the protrusion of radicle and plumule. Protein content was decreased in metal exposed plants at metal concentrations equivalent to those found in polluted soil (Rana Athar & Masood Ahmad 2002). Decrease in starch content with increasing concentration of mercury levels could be due to suppressed rate of starch synthesis. Soluble sugars decreased with increased concentration of mercury but the decrease was equal to control. Seed treatments were found to initiate higher mitochondrial activity leading to formation of more energy compounds (Henckel 1964). The reduction of soluble sugar could be due to suppressed amylase activity which is similar to finding of Prasad (1990) in rice. Mercury is known to have depressive effect on the metabolic pathway and energy generating process in seeds due to osmotic effects or ionic imbalance in metabolic process (Greenway & Munns 1980).

It may be concluded that the inhibition of germination, rate of germination and growth of seeds of *Vigna unguiculata* in response to mercury demonstrate that they suppress not only the protrusion of radicle or delayed the germination but also retarded the growth. The seedling growth is more sensitive to heavy metal (Hg^{2+}) in comparison to seed germination. Mercury treatment is potentially toxic to *Vigna unguiculata* root than shoot. Absorption depends on the ability of the root and may have an influence on synthesis of protein, carbohydrate, activity of enzyme and phenolic content some of which can be enhanced, while others get inhibited. The sensitivity of *Vigna unguiculata* seeds in terms of biochemical changes and enzyme activities was remarkably noticeable. The results suggest that biochemical changes and enzyme activity in *Vigna unguiculata* plants is promising indicator of heavy metal assay, which should be further explored so that its value can be evaluated when more data are available.

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