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EFFECT OF MERCURIC CHLORIDE ON SEED GERMINATION, SEEDLING GROWTH AND ENZYME ACTIVITIES IN MAIZE (*ZEA MAYS* L.)

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SUMMARY

The phytotoxic effect of mercuric chloride on seed germination and seedling growth of maize (var. Jaunpuri) was studied by seed soaking treatment with different concentrations of mercuric chloride (0 to 3.0 mM). Different physico-chemical parameters were studied, viz., seed germination, length of plumule and radicle, absolute water uptake (AWU%), relative water uptake (RWU%), amylase activity (endosperm) and proline content (seedling). Increase in concentration of mercuric chloride from 0.5 to 3.0 mM decreased seed germination significantly when compared with control. Similar trend was noticed in other parameters like radicle and plumule length, AWC%, RWU% and amylase activity. However, seedling proline content increased with increase in $HgCl_2$ concentration. In another experiment seed treatment for 48 h with mercuric chloride (0.5 to 1 mM), inhibited the no. of leaves, root length, shoot length, no. of adventitious root, leaf area, fresh weight of roots and shoot, chlorophyll and nitrogen contents and nitrate reductase activity while superoxide dismutase activity as well as membrane injury enhanced in 8 and 12 days old seedlings, respectively. However, values of no. of leaves, root length, shoot length and fresh weight of roots were not significant. Similarly, maize leaves collected from 12 days old control seedling when floated over mercuric chloride solution (0.5 to 1 mM) for 24 h a constant decline was noted in nitrate reductase activity whereas accelerations were recorded in superoxide dismutase activity and membrane injury. The finding suggested that the mercury has direct impact on membrane structure of the tissue and on the enzymatic activity of the plant system.

***Key words*: Absolute water uptake, mercuric chloride, relative water uptake, sulphyldryl**

INTRODUCTION

Among the heavy metals, mercury has drawn serious attention because of it's hazardous effect on environment. Mercury pollution in soil disturbs soil fertility status and causes phyto-toxicity to plants besides effecting human health. Plant exposed to mercury showed a reduction in photosynthesis, transpiration, chlorophyll synthesis and water uptake (Schlegel *et al.* 1987, Prasad and Prasad 1987). Reports also suggest that the mercury is taken-up by plant roots and then translocated and distributed in plant system (Covallini *et al.* 1999). Bose *et al.* (1983) had shown that mercury directly attacks on sulphydryl group of enzyme protease, partially purified from the endosperm during seed germination which brings the inactivation of this enzyme in maize. Seeds, the miniature of plant face a number of adverse conditions in soil ranging from heavy metal pollution to other abiotic stresses like salinity, alkalinity, water shortage etc during germination. These factors have a negative impact on seed germination and seedling growth of a particular plant / crop. Proline

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accumulation and an increase in superoxide dismutase enzyme activity during stress reflect the extent of adverse condition of the plant system (Hare and Cress 1997, Luna *et al*. 1994). However, the proline accumulation is also found to be influenced with heavy metal application (Alia and Saradhi 1991, 1993, Costa and Morel 1994, Schat *et al*. 1997). Kalimuthu and Shiva Subramanian (1990) showed an inhibition in the level of nitrogen, nitrate reductase activity and chlorophyll content with the application of mercuric chloride. However, studies related to the effect of different concentrations of $HgCl_2$ on plant metabolism particularly in maize are scanty. Hence, an experiment was carried in this direction to understand the effect of $HgCl_2$ at various doses on membrane structure and enzyme synthesis in maize var. Jaunpuri.

MATERIAL AND METHODS

Seeds of maize (*Zea mays* L.) var. Jaunpuri were procured from the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, BHU, Varanasi. After the sterilization of seeds with 1% solution of bleaching powder for 5 minutes, the seeds were washed thoroughly with distilled water and soaked for 6 h in different concentrations of $HgCl_2$ (i.e. 0.0, 0.5, 0.75, 1.0, 2.0 and 3.0 mM) and kept for germination for 48 and 96 h at 30±2 ^{0}C in incubator in controlled condition.

Observations regarding seed germination, length of root and shoot were taken up to 96 h. The seeds were considered to be germinated when the radicle became visible.

The absolute water uptake (AWU) and relative water uptake (RWU) of germinating seeds were recorded with the help of following formula.

$$AWU\% = [(\text{Fresh weight of seed - Dry weight of seed})/(\text{Dry weight of seed})] \times 100$$

$$RWU\% = [(\text{Fresh weight of seed - Initial weight of seed})/(\text{Initial weight of seed})] \times 100$$

The determination of the activity of amylase enzyme in endosperm, proline content, nitrogen content, nitrate reductase activity, superoxide dismutase and membrane injury in germinating maize seedlings/leaves were done by using methods of Bernfield (1955), Bates *et al*. (1973), Lang (1958), Srivastava (1974), Dhindsa (2000) and Blum and Ebercon (1981) respectively. Leaf area and chlorophyll content of maize seedlings were measured by using leaf area meter (model- 211) and chlorophyll meter/ spad meter (SPAD-502). All the experiments were repeated thrice. The experiments followed completely randomized design and statistical analyses were done as per requirement.

RESULTS AND DISCUSSION

Maize seeds soaked in different concentrations of $HgCl_2$ (0 to 3.0 mM) were tested for percent germination up to a period of 96 h. Germination (%) increased with time, however, as the concentration increased the percent germination decreased significantly. At 48 and 96 h durations, seeds treated with 3 mM $HgCl_2$ exhibited 31 and 45% germination against 68 and 95% in untreated sets (control), respectively. Pathak *et al* (1987) also observed inhibition in the percent germination of seeds treated with heavy metals (mercury and cadmium). Reduction in radicle and plumule length was also noticed (0.296 to 0.173 and 4.67 to 1.30 cm) due to increase in $HgCl_2$ concentration (0 to 1.0 mM) at the end of 96 h (Table 1). Similar reduction in radicle and plumule length due to heavy metals was reported by many scientists (Pathak *et al*, 1987; Sharma, 1985; Kalimuthu and Shiva Subramanian, 1990). Absolute water uptake (AWU%) and relative water uptake (RWU%) both showed a constant reduction with increasing concentration of $HgCl_2$ upto 3 mM in respect to control except at 96 h RWU% at 3 mM concentration was found to be more as compared to 2 mM concentration (Table 1).

$HgCl_2$ inhibited the activity of amylase increasingly with higher concentrations (Fig. 1). The study related to proline accumulation showed a constant increment in its level with increasing germination time and also with increasing concentrations of $HgCl_2$ up to 3.0 mM at both

Table 1. Effect of different concentrations of $HgCl_2$ on germination (%), length of plumule/ radicle (cm), absolute water uptake (AWU %) and relative water uptake (RWU %) in maize seeds.

Parameters		Concentrations of $HgCl_2$ (mM)						C.D. at 5%
		0.0	0.50	0.75	1.0	2.0	3.0	
Germination (%)	48 h	68	58	60	53	36	31	2.6082
	96 h	95	91	88	88	63	45	3.8542
Length of Plumule (cm)	48 h	0.066	0.053	0.036	0.033	0.023	0.026	0.0381
	96 h	0.296	0.266	0.213	0.203	0.183	0.173	0.0313
Length of radicle (cm)	48 h	0.75	0.49	0.36	0.32	0.19	0.18	0.3339
	96 h	4.67	4.40	4.05	3.30	1.86	1.30	1.1235
AWU (%)	48 h	45.6	43.3	33.6	36.0	30.0	28.0	10.594
	96 h	43.0	33.3	31.0	30.3	30.6	23.3	10.842
RWU (%)	48 h	29.6	28.0	17.3	15.2	14.3	12.3	9.293
	96 h	28.0	28.0	23.7	16.3	9.0	10.5	13.57

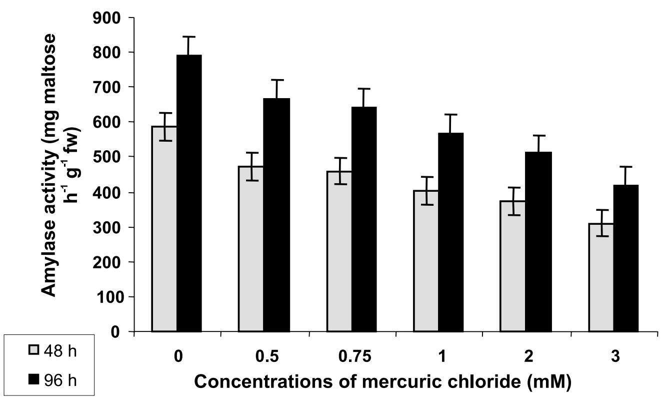

Fig. 1. Effect of different concentrations of $HgCl_2$ on amylase activity in germinating endosperm of maize seeds at 48 h and 96 h

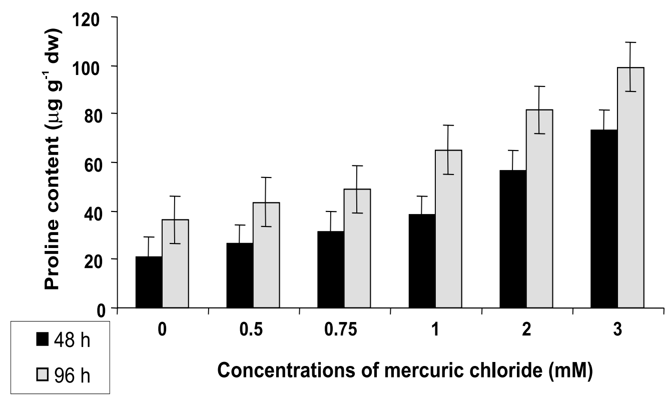

Fig. 2. Effect of different concentrations of $HgCl_2$ on proline content in germinating seedlings of maize at 48 h and 96 h

the periods (Fig. 2). Mishra and Chaudhary (1997) reported that heavy metals (like lead) inhibits the germination and seedling growth of rice cultivars by inhibiting the hydrolysis of endosperm starch. Cadmium inhibited the activities of α and β amylase in germinating pea seeds (Chough and Sawhney, 1996). In the present investigation studies on amylase clearly indicated that the inhibition of germination by $HgCl_2$ is directly regulated by the activity of starch hydrolyzing enzyme. In the presence of $HgCl_2$ the proline accumulation increased in germinating seedlings. Proline has been reported to accumulate in plants in response to a wide range of environmental stresses (Hare and Cress, 1997). Shaw and Rout (2002) reported that $HgCl_2$ and $CdCl_2$ not only enhance the proline accumulation but also increase the key proline biosynthesizing enzyme pyrroline corboxylate synthetase in germinating seedlings of *Phaseolus aureus* and in *Triticum aestivum*. The present study is in support of the above mentioned findings.

Sharma (1985) noticed heavy metal induced inhibitory effect on mobilization of N and P during germination and seedling growth. Bose *et al*. (1983) reported that parachloromercuro benzoate inhibit the activity of partially purified protein hydrolyzing enzyme protease via replacing enzyme-SH bond by mercury. On the basis of present investigation, it may be predicted that the mercury during soaking enters to the seed and inhibits the activity of hydrolyzing enzymes by replacing their SH

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group, as a result enzymes become inactive and are unable to hydrolyze the stored materials, which in turn may reduce the translocation of food reserves towards growing embryo from endosperm. It is reported that mercury inhibits various metabolic functions by replacing SH group from enzymes (Chrispeels and Agre, 1994).

The effect of $HgCl_2$ treatment on seeds for 48 hrs was studied on various physiological and biochemical parameters of 12 day old seedlings (Table 2 and 3). The higher concentrations (2.0 and 3.0 mM) of $HgCl_2$ were discarded in this part of study due to their severe inhibitory effects on germination (Table 1). Result revealed that no significant effect of treatment on root length, shoot length and number of leaves was found (Table 2). Number of adventitious root reduced with increasing concentrations of $HgCl_2$. Observation further showed reduction in leaf area with the higher

Table 2. Effect of pre-soaking treatment (48 h) to seeds with different concentrations of $HgCl_2$ on some growth parameters in 8 days old maize plants

Conc. of $HgCl_2$ (mM)	No. of leaves/ plant	Root length/ plant (cm)	Shoot length/ plant (cm)	Number of adventitious root /plant	Leaf area (cm^2)	Fresh wt. of roots/ five plants (mg)	Fresh wt. of shoot/ five plants (mg)
0.0	2.7	12.3	12.3	7.0	37.5	1166	7.16
0.5	2.7	7.3	9.6	4.6	36.2	1366	6.73
0.75	2.0	6.8	9.8	2.0	18.3	1033	4.30
1.0	2.0	6.8	8.3	3.3	15.4	883	4.00
C.D. at 5%	N.S.	N.S.	N.S.	2.28	5.468	N.S.	1.954

Table 3. Effect of different concentrations of $HgCl_2$ on chlorophyll content (SPAD unit), nitrogen content (mg g^{-1} dw), nitrate reductase (nmol NO_2^- g^{-1} fw h^{-1}), superoxide dismutase (unit $min^{-1}g^{-1}$ fw) activity and membrane injury (% electrolyte leakage) in maize seedlings.

Parameters	Days after sowing	Control	Concentrations of $HgCl_2$ (mM)			C.D. at 5%
			0.5	0.75	1.0	
Chlorophyll content	4	22.8	20.0	17.3	16.2	3.80
	8	23.3	21.0	20.8	19.6	3.090
	12	24.9	17.6	16.5	16.2	6.2 00
Nitrogen content	4	14.8	12.4	11.9	9.9	2.588
	8	16.1	15.3	14.7	12.5	2.565
	12	20.8	13.6	12.8	10.2	2.195
Nitrate reductase	4	319	247	142	139	16.44
	8	370	299	252	230	119.06
	12	405	262	239	224	18.059
Superoxide dismutase	4	18.29	26.18	35.46	37.34	4.187
	8	41.64	69.26	74.10	85.01	6.700
	12	51.22	73.37	79.33	95.24	6.553
Membrane injury	4	76.9	86.8	87.3	90.0	8.59
	8	80.0	86.4	87.5	94.4	4.25
	12	81.1	84.3	83.3	94.8	5.31

*DAS=Day after sowing

concentration of $HgCl_2$ as compared to control. The lowest concentration (0.5 mM) improved the fresh weight of root in comparison to control but the rest of the concentrations (0.75 to 1.0 mM) showed an inhibitory effect. Shoot fresh weight inhibited at all concentrations of $HgCl_2$ in comparison to control (Table 2). Bharti and Singh (1993) reported that the fresh weight of growing leaves and roots were inhibited with lead in *Sesamum indicum* cv P B-1. Cadmium was also found not only to inhibit the growth of maize leaves and its roots but also decreases the leaf weight (Prasad 1995). Samantharay *et al*. (1998) found that the root growth of Italian millet was affected by the use of various heavy metals.

The chlorophyll content decreased significantly with increasing concentration of $HgCl_2$. It was found 17.6 SPAD at 0.5 mM concentration which gradually decreased with higher concentration i.e. 0.75 mM (16.5 SPAD) and 1.0 mM (16.2 SPAD) in respect to control (24.9 SPAD) (Table-3). Stobard *et al*. (1985) suggested that the activity of enzymes involve in the synthesis of chlorophyll were inhibited with use of heavy metals which directly influence the biosynthesis of chlorophyll. Prasad and Prasad (1987) while testing the effect of Hg in *Pennisetum typhoides* observed a significant reduction in the activity of α-amino levulinic acid dehydrogenase.

Nitrogen content in 4 days old seedlings decreased slowly with increasing the concentrations (0.5 to 1.0 mM) of $HgCl_2$. Significant reduction in nitrogen contents was observed at 1.0 mM concentration (33.1, 22.36 and 50.96%) as compared to control at 4, 8 and 12 days old seedlings respectively (Table 3). Nitrate reductase increased with increasing age of the seedling in control from 4 to 12 days. However, application of $HgCl_2$ showed a negative impact on the activity of enzyme with increasing concentration. Severe inhibitory effect was noticed in 12 in comparison to 8 days old seedlings of maize and their control, respectively (Table 3). The study on nitrogen metabolism in respect to nitrogen content and NR activity showed that both were declined with increasing concentration of $HgCl_2$. Kalimuthu and Shiva Subramanian (1990) reported that the protein and amino acid contents of *Zea mays* variety Co-1 when treaded with $HgCl_2$ solution showed a gradual inhibition in protein and amino acid contents in seedlings from 22-200 µg/ml $HgCl_2$.

Superoxide dismutase was measured in the leaves obtained from the 4, 8 and 12 days old maize plant. At 4 DAS, the increment in the SOD activity with increasing concentration of $HgCl_2$ was noted to be less as compared to latter stage of the study i.e. at 12 DAS in respect to control. Luna *et al*. (1994) observed that the activity of SOD enhanced by incubating 6 and 9 days old seedlings in a solution containing copper ion and suggested that copper which causes breakdown of chlorophyll and carotenoid also causes an increase in membrane permeability and lipid peroxidation. Mercury might have caused, in present study the same effect which resulted a decrease in chlorophyll content in leaves with a correlated increase in membrane leakage (Table 3).

Table 4. Effect of different concentrations of $HgCl_2$ on membrane injury (% electrolyte leakage), nitrate reductase (nmol NO_2^- g^{-1} fw h^{-1}) and superoxide dismutase (unit $min^{-1} g^{-1}$ fw) activities in leaves collected from 12 days old maize plants and floated over $HgCl_2$ solution of different concentrations (0, 0.50, 0.75 and 1.0) for 24 h.

Parameters	**Control**	**Concentrations of $HgCl_2$ (mM)**			**C.D. at 5%**
		0.5	**0.75**	**1.0**	
Membrane injury	92.5	92.81 (0.33)**	93.88 (1.49)**	98.86 (6.87)**	2.92
Nitrate reductase	394	298 (-24.24**)	187 (-52.27)**	88 (-77.66)**	15.78
Super oxide dismutase	48.1	74.12 (54.09)**	82.71 (71.95)**	97.8 (103.32)**	10.54

**=Percent Change

The membrane injury was measured in terms of percent electrolyte leakage. The membrane injury increased with increasing concentration of $HgCl_2$ as well as with the age of seedlings. Severe injury i.e. 90.0, 94.4 and 94.8 % was found at 1.0 mM concentration of $HgCl_2$ in comparison to control i.e. 76.9, 80.0 and 81.1% for 4, 8 and 12 DAS respectively. Thompson (1988) and Hendry *et al*. (1992) suggested that this may be due to de-esterification of membrane lipids. The study on superoxide dismutase showed increase in activity with the increase in the concentration of $HgCl_2$. This may be due to an increase in the heavy metals stress on the plant tissue which also causes membrane injury (Table 3).

To see the direct role of $HgCl_2$ on tissue system, 12 days old secondary leaves of maize plant were floated either in the distilled water (control) or in different concentrations of $HgCl_2$ (0.5 to 1.0 mM) for 24 h. The activity of NR enzyme was found to be declined with increasing concentration $HgCl_2$ in respect to control (Table 4). The percent reduction with 1.0 mM $HgCl_2$ treatment was 77.66%. However, regarding membrane injury as the concentration of $HgCl_2$ increased the leakage of electrolytes also increased. Percent electrolyte leakage was found 98.86% in 1.0 mM $HgCl_2$ treatment in respect to control whereas, 103.33% increment was noticed in case of SOD activity in comparison to control (Table 4). Thus, Superoxide dismutase enzyme also represented the same trend. Hence, it is noticed from present findings that mercury may play a role in the membrane injury of the tissue which in turn may disturb the uptake of water as well as nutrients which reflect an alteration in normal metabolic processes.

Present investigation suggests that seed germination in presence of mercury affects post germinating phases of plant growth. This may be acted via the root growth of the plant because from the present data, it is observed that mercury not only inhibits the root length of maize plant, but also reduced the number of adventitious roots. It is obvious that when in any plant root system, affected by any means, the uptake of the nutrient would be affected very adversely. Consequently, the plant growth above the ground parts is also affected. Further, the direct application of $HgCl_2$ to the leaves, suggested that the $HgCl_2$ directly causes an injury to the membrane and inhibits the enzyme of nitrogen metabolism and thus creates a stressful situation within the system which leads to increase in SOD activity.

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