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STUDIES OF MERCURY ($HGCL_2$) STRESS ON MORPHOLOGY AND PIGMENTS OF *VIGNA RADIATA* L.

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ABSTRACT

Green gram is considered as the life line of man but it has been facing various threats from increase in use and toxic effects from the chemicals. The million dollar question is how to strike out a perfect balance between these two to minimize the chemical effect while retaining its maximum yield to meet daily demands. In the event of this stalemate, the present study takes up green gram (*vigna radiata*) production using safe level of chemical fertilizer mercury. Apart from being a highly essential food with nutrients required for the formation of body, the legumes of the plant act as a vital cog in the wheel of nitrogen fixation indispensable for preserving the soil fertility. In a sense, it is a blessing in disguise for the mankind in more than one ways. The laboratory experiment is carried out on the actual intensity the actual intensity of the chemical on various levels of seedlings for seven days. The percentage of germination and leaf emergence decreases gradually, and finally, 40% and 74% o decrease was recorded when the concentration is the highest at 1000mg/L. The value of % change in root and shoot length is highest in control(100%) and subsequently decreases, finally 78% and 84% decrease is recorded at 1000 mg/L The ratio of root and shoot length in control or untreated seeds is 1.63 cm and it increases at 800 mg/L is 5.8 cm and finally at 1000mg/L concentration it is 2.2 cm The decreasing level of fresh and dry weight was observed in both root and shoot of the seedling level of$HgCl_2$ concentration. The contents of chl-a, chl-b,total chl of the control seedlings in the shoots of 7 days old seedling were 3.057,4.599,7.645mg/g fresh weight respectively. The amount of chl-a,chl-b and total chl of the exposed seedlings of 1000mg/l has shown a fall by 0.79,0.826,1.615 respectively.

KEYWORDS: *Vigna radiata (*,green gram*)*, $HgCl_2$ (Mercuric chloride) mercury tolerance, nitrogen fixation, ecological crisis, heavy metals.

INTRODUCTION

Day to day demands of man has increased in many-fold terms to seek new avenues of production, which is evidently scarce in supply, that calls upon research on how to grow more to feed millions for their very survival.

Green gram (*Vigna radiata*) is one of the most highly prized pulses in tropical countries, especially in India. The green pods are eaten as vegetable and they are highly nutritious. Green legumes play a vital role in improving soil health through biological Nitrogen fixation and are cheapest source of dietary protein for human and livestock. Their yield is low and unstable due to water stress during growth stages.

Industry released toxic substances in to soil., water, atmosphere, forest and in to the human food chain symbolized by the LOS angles smog, and proclaimed "death" of the lake Eris and the progressive pollution of all major rivers of the world. Heavy metal stress causes multiple direct and indirect effects on all physiological processes of plants. All parameters exhibited reduced trend with increase in $HgCl_2$ concentration when compared to control plants. Annual production of mercury in the world is estimated to be about 9000 tons and about 50% of its lost to the environment as pollutant (Goldberg, 1976). The most common heavy metals contaminate Lead(Pb), Cadmium(cd) ,Chromium(Cr), Copper(Cu), Mercury(Hg), Nickel(Ni) and Zinc(Zn) due to the awareness of the pessimistic effect of ecological pollution, everyone is appropriately aware about innovative methods for preventing pollution of the environment including soil.

The first major incident involving mercury poisoning in human was reported from Minamata bay, Japan. Eating contaminated fish caused the disease of the central nervous system, now known as "Minamata disease". The methyl mercury contamination was a result of water discharges from an acetaldehyde plant. Fish and shellfish concentrated mercury to the levels of 10 mg kg^{-1} (Anonymous, 1972). They can create major ecological crisis, since they are conserved in nature. They often accumulate in plant parts and biologically magnified through tropic levels. es. Heavy metals are integral components of ecosystem.

The uptake and excretion of Mercury have been studied in seedlings of woody plants(Kotov, 1983), in plant shoot of oats and lettuces(Staiger, 1983), in theeelgrass Zostera marina(Lyngby and Brix, 1982), in crops grown on sludge treated soil. Mercury has been shown to be released from sediments and accumulated by plants like *Ceratophyllum demersum* and *Anodontagrandis* (Hammer*et al*., 1998). Toxicity of mercury has been reported in many plants and very low concentrations of mercury causes hazards to plant growth (Vallee and Ulmer, 1972; Sandmann and Boger, 1983; Kagi and Hapke, 1984; Baker et al., 1985; De et al., 1985). According to Goldberg (1976), maximum consumption of Mercury is in the chlor-alkali plants. The products of chlor-alkali industry can also be source of long term mercury pollution. House-hold bleaching solutions contain 17-24 ppb of Mercury (Siegel and Eshleman, 1975). Studies of micronutrient requirements and toxic effects of trace metals on soil organisms and native plants in field are limited. Usually the effect of metals is examined in sterile and much simplified laboratory conditions in different degrees (Ross, 1994). According to Goldberg (1976), maximum consumption of Mercury is in the chlor-alkali plants. The products of chlor-alkali industry can also be source of long term mercury pollution. House-hold bleaching solutions contain 17-24 ppb of Mercury (Siegel and Eshleman, 1975). Mercuric chloride is used as catalysts in the manufacture of plastics and acetaldehyde, and effluents from such plants contribute mercury into the aquatic environment. Mercury is also used in the production of batteries, street lamps, fluorescent tubes, circuit breakers etc., all of which are finally discarded as waste. Mercury compounds are used in anti-fouling paints, pulp and paper industry and are lost in the waste water effluents. Phenyl mercuric acetate and ethyl mercuric chloride have been used as fungicides. According to Kalimuthu and Sivasubramanian (1990), the percentage of germination, root and shoot length of maize seedlings decreased with increasing concentration of lead and mercury.

Fossil fuel burning and cement manufacturing cause emission of mercury into the atmosphere (Syamala and Rao, 1999). Sewage disposal contributes to mercuric contamination of aquatic environment. Mercury may get remobilized by burning of these sledges or when used as fertilizers (Moore and Ramamoorthy, 1984). Puerner and Siegel (1972) studied the toxic effects of mercury compounds in the growth and orientation of cucumber seedlings and noticed that mercury with fluorescein affected not only the orientation of cucumber seedlings but also the toxicity of the Hg ions itself. Mercuric chloride was found to be more toxic than fluorescein indicating that the combination of mercury with fluorescein can mitigate metal toxicity.

MATERIALS AND METHODS

Test organism

The seeds of green gram (*Vigna radiata*) of variety OBGG -52 Durga, were procured from the pulse Research Institute (CPRI) Ratanpur, Berhampur. The seeds with uniform size, colour and weight were chosen for experimental purpose. *Vigna radiata L*. Is a perennial legume of family Fabaceae and a short duration variety.

Selection of toxicant

Mercuric chloride was used in this work for HIMEDIA. First stock solution of 1000mg/l wa s prepared in the following proportion, i.e 1gm of $Hgcl_2$ in 1 lit. Distilled water. Subsequent dilutions were made using distilled water and stock solution of control, 200, 400, 600, 800, 1000 mg/L. Fresh test solutions were prepared each time experiments were performed.

Parameters evaluated

The seedlings parameters studied were root length, shoot length and pigments(chlorophyll a, chlorophyll b,totalchlorophyll**,** carotenoid, phaeophytin) (Arnon,1949) of the seedlings growth period of 7 days. The growth of plants was evaluated by measuring the shoot and root length of seedling on the 7^{th} day. In this morphological study, three plants were taken and the mean was also calculated. 15cm scale was used for the measurement of the root and shoot length. Finally R/S ratio was also recorded. Again the fresh weight of root and shoot were measured by weighing balance machine and the same are kept in an oven for a whole night at 50^0C. Then measured the dry weight of root and shoot were observed by weighing balance machine. Fresh leaves of 200mg weight taken and grinded in 80% acetone (vol/vol) in a mortar and pestle. Then the extract was taken in centrifuge tubes and centrifuged (3000rpm for 10 minutes) Then the supernatant were collected and 5ml of 80% acetone added to the residues, stirred and centrifuged again for 10 minutes. The volume of the supernatant was made up to 10ml and absorbance of each extract was determined by using spectrophotometer at the wavelength of 475, 550, 570, 580, 645, 663, 665, 666nm against80% acetone.

Chlorophyll-a, chlorophyll-b and the total chlorophyll content was measured by recording the absorbance of the extract at 645 and 663nm wavelength and the values were calculated by using the formula given by Arnon (1956). The carotenoid content was quantified by recording the absorbance of the extract at 475 and 550nm. The phaeophytin content was measured by recording the absorbance of the extract at 665 and 666nm and the values were calculated by using the formula given by Vernon (1960).

RESULTS

Germination studies

The maximum germination and leaf emergence was observed in control. The percentage of germination and leaf emergence decreases gradually, and finally, 40% and 74% o decrease was recorded when the concentration is the highest at 1000mg/L. The changes in leaf emergence of 7th days old seedling were shown in Table-1 and Fig 1. The value of % change in root and shoot length is highest in control(100%), and subsequently decreases, finally 78% and 84% decrease is recorded at 1000 mg/L. The results obtained were presented inTable-2 Fig-2. **R/S Ratio**: The ratio of root and shoot length in control or untreated seeds is 1.63 cm and it increases at 800 mg/L is 5.8 cm and finally at 1000mg/L concentration it is 2.2 cm. The result obtained were presented in Table-3 and Fig-3.

Fresh and dry weight: The maximum weight of fresh root and shoot was observed in control (0.153 and 1.977mg respectively) and weight of dry root and shoot in control (0.05 and 0.227mg respectively). The decreasing level of fresh and dry weight was observed in both root and shoot of the seedling level of$HgCl_2$ concentration. The result obtained were presented for fresh and dry weight of root and shoot in Table-4 and Fig-4.

PIGMENT CONTENTS

The contents of chl-a, chl-b, total chl of the control seedlings in the shoots of 7 days old seedling were 3.057,4.599,7.645mg/g fresh weight respectively. The amount of chl-a, chl-b and total chl of the exposed seedlings of 1000mg/l has shown a fall by 0.79,0.826,1.615 of respectively. chemical. The carotenoid content of control seedlings in the shoot was 0.66mg/g fresh weight. There was decrease in carotenoid content with increase in $Hgcl_2$ concentration. The carotenoid content was about 0.206 in 1000mg/L of Hg stress as compared to control. Phaeophytin content of control seedlings in the shoot was highest value i.e 29.949mg/g fresh weight and it decreases i.e, 2.569mg/g in 1000mg/L. There was decrease in phaeophytin content with increase in concentration. There was decrease in chl-a, chl-b, total chl, carotenoid, phaeophytin content with increase in the concentration of $HgCl_2$. Pigments viz chl-a, chl-b, total chl, carotenoids and phaeophytin contents were present in Ttable-5 and Fig-5.

Table-1 -Effect of different concentrations of $HgCl_2$ on the percentage germination and leaf emergence of *Vigna radiata* seedling.

Concentration of $HgCl_2$ Mg/L	% of germination	% Leaf emergence
0	100	100
200	93	86
400	86	66
600	83	66
800	76	40
1000	60	26

Table- 2: Effect of different concentration of $HgCl_2$ on the root length (in cm) and shoot length (in cm) of 7-days old *Vigna radiata* L. Seedling.(Values in parentheses indicate the percentage change from control value).

Concentration of $HgCl_2$ Mg/L	Root Length in Cm.	Shoot Length in Cm.
0	9.0 (100)	5.5 (100)
200	8.7 (96.66)	6.2 (112.72)
400	8.0 (88.88)	6.5 (118.18)
600	5.5 (61.11)	2.0 (36.36)
800	2.9 (32.22)	0.5 (9.09)
1000	2.0 (22.22)	0.9 (16.36)

Table-3: Effect of different concentration of $HgCl_2$ on the ratio of root and shoot length(in cm) of 7-days old *Vigna radiata* L. Seedlings.

Conc of $Hgcl_2$ mg/L	0	200	400	600	800	1000
R/Sratio	1.63	1.40	1.23	2.73	5.8	2.3

Table-4: Effect of different concentration of $HgCl_2$ on the percentage of root and shoot fresh weight of 7-days old *Vigna radiata* L. Seedlings.(Values in parentheses indicate the percentage change from control values.)

Concentration of $HgCl_2$ mg/L	Fr.wt in mg (shoot)	Fr.wt in mg (root)	Dry.wt in mg (shoot)	Dry.wt in mg (root)
0	1.977 (100)	0.153 (100)	0.227 (100)	0.05 (100)
200	1.484 (75.06)	0.098 (64.05)	0.205 (90.30)	0.038 (76)
400	0.638 (32.27)	0.001 (0.65)	0.15 (66.07)	0.031 (62)
600	0.655 (33.13)	0.018 (11.76)	0.132 (58.14)	0.03 (60)
800	0.291 (14.71)	0.09 (58.82)	0.113 (49.77)	0.007 (14)
1000	0.127 (6.42)	0.035 (22.87)	0.062 (27.31)	0.035 (70)

Table-5: Effect of different concentration of $HgCl_2$ on the pigment contents(mg/L) of 7-days old *Vigna radiata* L. Seedlings.

Conc.	Chl.a	Chl.b	Total chl.	Carotenoid	Phaeophytin
0	3.057	4.599	7.645	o.66	29.949
200	3.549	5.27	8.810	0.73	18.98
400	3.371	3.995	7.839	0.717	11.26
600	2.879	3.211	6.088	0.708	9.87
800	1.873	2.048	3.920	0.449	6.544
1000	0.79	0.826	1.615	0.206	2.569

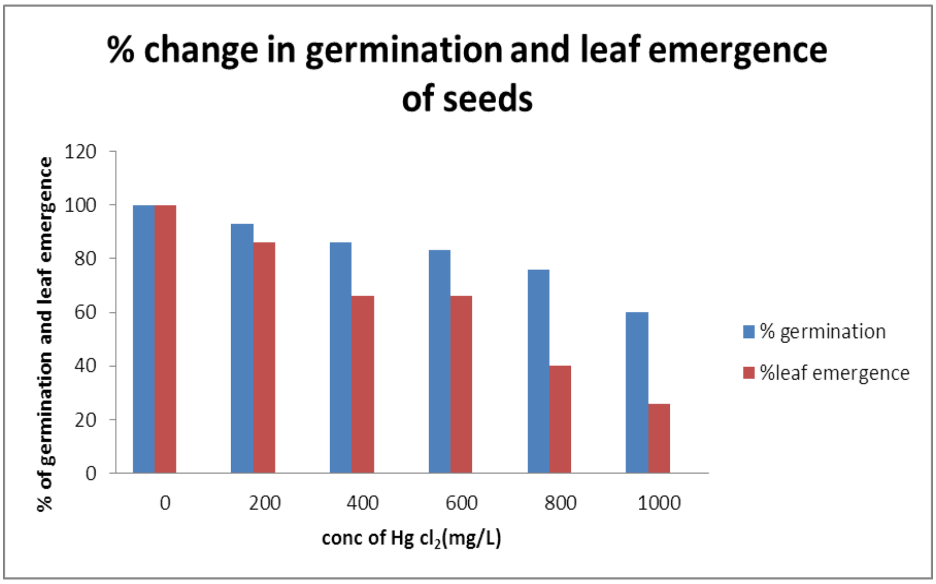

Figure-1: Percentage change in germination of seeds and leaf emergence with respect to control of *Vigna radiata* L.seedling treated with different concentrations of HgCl2.

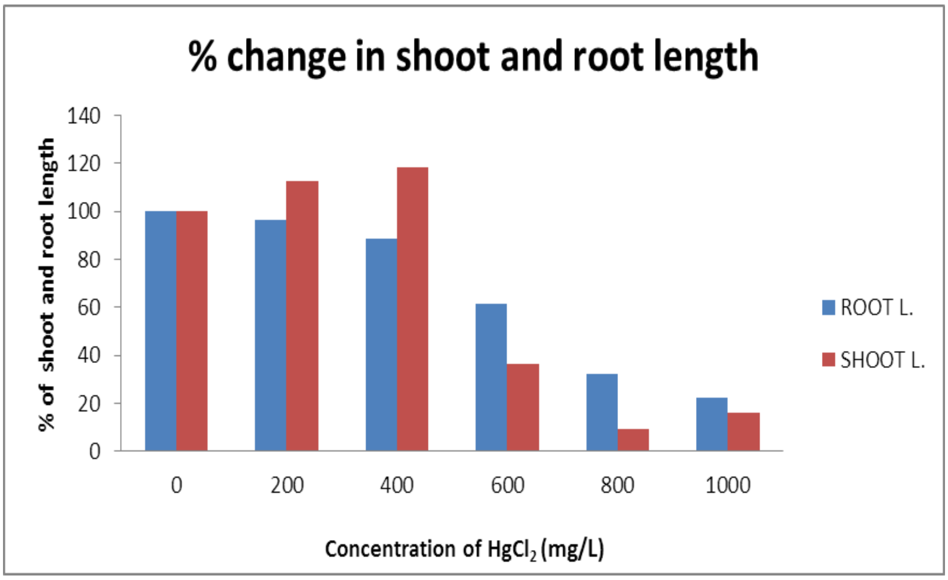

Figure: 2 The Percentage change in shoot and root length with respect to control of *Vigna radiata* L.seedling treated with different concentrations of $HgCl_2$.

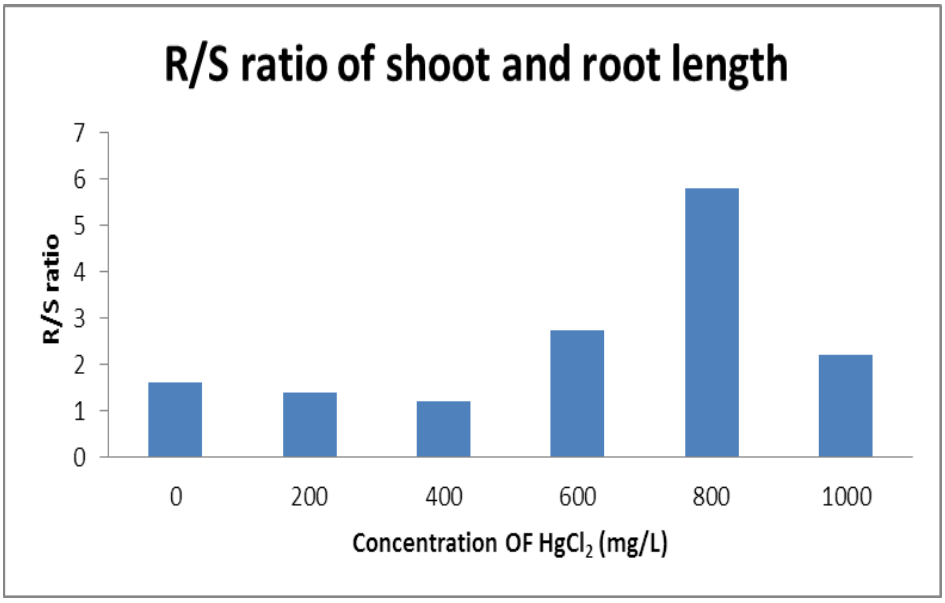

Figure- 3: The graph showing percentage change of ratio of shoot and root length(in cm) with respect to control of *Vigna radiata* L. Seedlings treated with different concentrations of $HgCl_2$.

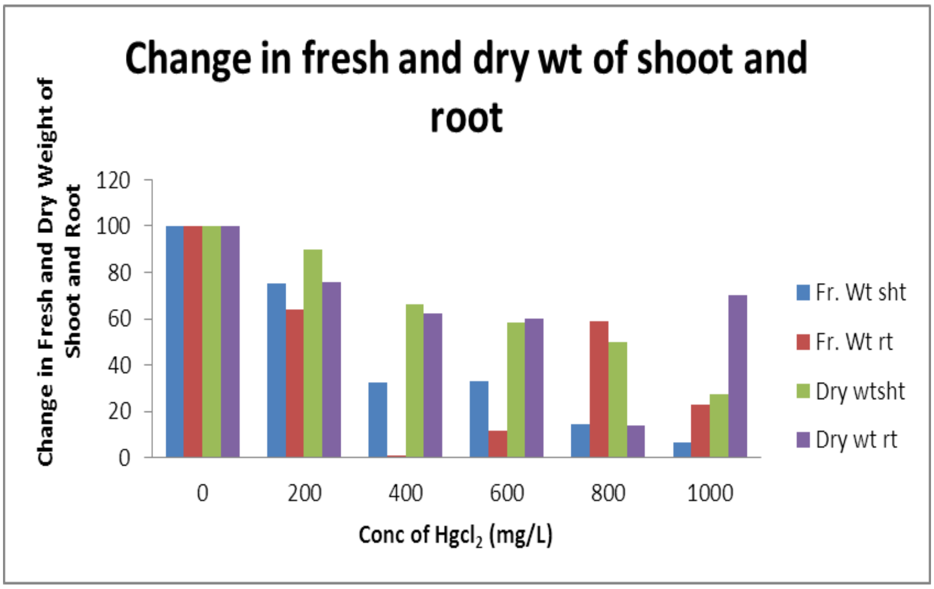

Figure 4: The graph showing percentage change of fresh weight of shoot and root length(in cm) with respect to control of *Vigna radiata* L. Seedlings treated with different concentrations of $HgCl_2$.

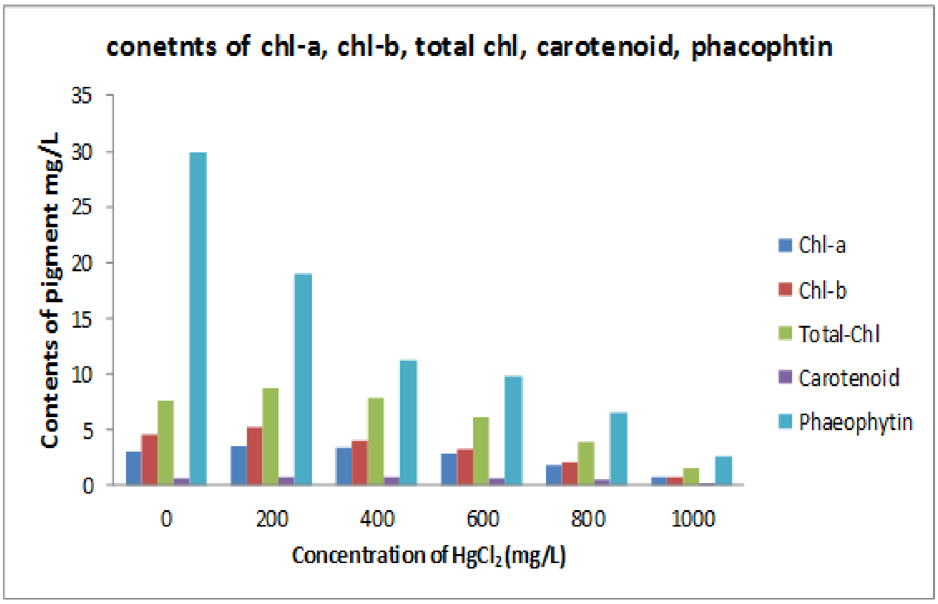

Figure-5: The graph showing change in total chlorophyll, carotenoid and phaeophytin contents in the shoots of 7th old *vigna radiata* L. Seedlings exposed with different concentrations of $Hgcl_2$.

DISCUSSION

Pollution of the environment with toxic substance has been the subject of interest for many researchers. Toxic substances are not easily defined since the effect of exposure to any potentially poisonous material depends on both its concentration and duration of exposure. Heavy metals can find their way into leaves either through growth in medium or through atmosphere.

Pratima *et al.,* has reported the effect of mercuric chloride on growth and biochemical constituent of green gram. Pathak *et al.,* (1987) has reported the first visible damage due to HgCl2. It inhibit germination when increase concentration of $HgCl_2$, also reduced length of root and shoot.$HgCl^2$ applications had positive effect on plant growth. The lower zinc applications, improved the root system of plants and it may helped the plants to the better absorption of water and other nutrients dissolved in the soil.

In general, decrease in germination has been one of the important manifestations of metal toxicity (Mukhia *et al.,* 1983). There are reports of phytotoxic effect of mercuric chloride on seed germination and seedling growth of maize(var. Jaunpuri) was studied by seed soaking treatment with different concentration of mercuric chloride(0 to 3.0 nM). Exposure to Hg can also reduce photosynthesis, transpiration rate, and water uptake and chlorophyll synthesis.

Both organic and inorganic Hg have been showed to cause loss of potassium, magnesium, manganese and accumulation of iron. These decreases explain the change in the permeability of cell membrane by compromising its integrity.

CONCLUSION

Among heavy metals, mercury is one of the most common toxic substances in the environment. It comes from various environmental sources viz., agricultural land which is influenced by anthropogenic activities, excessive use of agrochemicals, fertilizers and also found in atmosphere as dusts, vapours fumes, water and in soil as a mineral. Mercury readily enters into the food chain and can subsequently affect the human and animal health.

The germination data showed that there is direct impact of concentration of mercury on the germination of seeds. When plants are exposed to $HgCl_2$ stress at high concentration inhibit seed germination, the growth and development of seedlings occurs. There are found that the negative impact of dose of mercuric chloride on the germination of seeds. At a higher concentration i.e., 1000mg/l of toxicant, the seed germination is very less than that of control. Both root and shoot growth of seedling had bad impact with exposed to high concentration of mercuric chloride. Roots were high affected and much more reduced than that of shoots .Morphologically, they look different from normal roots by their colour, shape and size. Plants can be exposed to mercurials either by direct administration as antifungal agents, mainly to crop plants through seed treatment or foliar spray, or by accident. The end points screened are seed germination, seedling growth, relative growth of roots and shoots. The absorption of organic and inorganic mercury from soil by plants is low and there is a barrier to mercury and translocation from plant roots to tops. Mercury affects both light and dark reaction of photosynthesis. Effect of different concentrations of mercuric chloride was visible in different pigment concentration of leaves. With increase in the concentration of the toxicant expose to the seeds which showed a decline in chlorophyll-a, chlorophyll-b, total chlorophyll, carotenoid and phaeophytin contain in shoots. This was a clear indication of fall in the plant growth rate and pigment content had direct impact on photosynthesis.

Conclusively, our result show that Mercury at higher concentration decreases seed germination, Chl-a, Chl-b, total chlorophyll, carotenoids, phaeophytin. This results suggests that the presence of mercury at higher concentration caused in growth inhibition, a decline in physiological and pigment activities, structural damage etc. Minimum use of mercury containing compounds in fungicide, pesticide and nematicide is recommended. Special care should be taken to monitor the toxic pollutants available in the immediate environment. The accumulation of such type of toxic pollutants in larger concentrations by crop can produce harmful effect to crops and ecosystem as well.

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