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ASSESSMENT OF HEAVY METALS MERCURY AND CADMIUM TOXICITY ON GERMINATION AND SEEDLING GROWTH OF *CAJANUS CAJAN* (PIGEON PEA)

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ABSTRACT:- The present study was carried out to test the toxic effect of heavy metals mercury chloride and cadmium chloride on germination of seedling growth, shoot length and dry biomass of and of *Cajanus cajan L.* was evaluated under laboratory conditions. The seeds of *Cajanus cajan L*. were germinated in different concentrations of (0, 15,10,20,50 mg/L) mercuric chloride and cadmium chloride. The germination parameters like germination percentage root and shoot length, fresh and dry weight of 10 days seedlings showed a decreasing trend with the increase in concentration of mercuric chloride and cadmium chloride. Hg and Cd showed significant inhibition of root elongation and the inhibition was more pronounced in the seedlings treated with cadmium.

KEYWORDS: - Mercury, *Cadmium*, *Cajanus* cajan, Seedlings.

INTRODUCTION:-

Chemical waste resulting from industrial activities and agricultural effluents such as fertilizer, herbicides and pesticide has contributed to the increased accumulation of heavy metals in soil (Fu et al. 2014; Hu et al. 2013; Nicholson et al. 2003; Neilson and Rajakaruna 2015). Since heavy metals are non-degradable, they can cause long-term deleterious effects on ecosystem health. Heavy metals are the natural components of the earth crust and are present in soil, water and living matter. Elevated levels of heavy metals due to anthropogenic activities such as extended use of superphosphate fertilizers, sewage discharge, industrial effluents and smelters dust spreading cause heavy metal pollution. The uptake and accumulation of heavy metals by plants is hazardous, since plants are part of the food chain. Contamination of food supplies by heavy metals may lead to risk for human and animal health. Most studied aspect of heavy metal toxicity is the damaging effect on seed germination and seedling growth of different plant species.

Soil contains different heavy metals. Metals are continuously released to the soil by volcanoes, natural weathering of rocks and industrial activities such as mining and combustion of fossil fuel, urban waste disposal, surface run off. They present a risk for primary and secondary consumer and ultimately to human beings. Excess accumulation of heavy metals operate as stress factors causing physiological constraints leading to decreased vigour and plant growth. Heavy metals cause pollution after reaching certain concentration in soil. Mercury and cadmium has no essential function but cause toxicity above certain tolerance level. Recently, there was a great concern for mercury as an environmental pollutant. Mercury occurs in organic as well as in inorganic forms and both the forms are highly toxic. Extensive use of mercury containing compounds, fungicides, algaecide, paper pulp industries and in agriculture, create a problem of mercury concentration in soil and water. Cadmium is considered to be one of the most toxic metal.

Cadmium-

Cadmium compounds are water soluble. It is used for dye and paint, pigment production batteries, ceramic industries, wooden industries and plastic production. The metal is also used in electroplating, semi-conductor, rectifiers, solder for aluminium etc. Industries that are engaged in extraction, refining, electroplating and welding of Cadmium containing materials and also refining copper, lead and zinc etc. are the chief sources of cadmium. Cadmium is the chief constituents of Phosphatic fertilisers. Application of high levels of commercial fertilisers increases cadmium level in the soil. It would accumulate in the soil permanently and also in plant products. Plants uptake is one of the major pathways by which toxic metals enter to human food chain. Heavy metals presence in the atmosphere, soil and water even in trace concentration can cause serious problems to all living being (Chandra Sekhar et al., 2011). Accumulation and exclusion were two basic strategies by which plants respond to elevated concentration of heavy metals (Mikus et al. 2005). Treatment of high concentration of some heavy metals to plants shows in reduction of seed germination and seedling growth (Patra and Sharma 2000, Peralta et al. 2001, ‘ Abedin and Meharg 2002, Jeliazkoava et al. 2003, Shafiq and Iqbal 2005, Marchial et al. 2006, Marques et al. 2007, Ashraf and Ali 2007, Hussain et al. 2007, Mahmood et al. 2007, Singh Sengar et al. 2008, Jun et al. 2009, Milian et al. 2011). Mercury pollution has become an important current issue of its environment (Sharon et al. 2012). Seed germination was significantly decreased with mercury treatment. The effect of mercury chloride was found to be highly toxic to seedling growth of Archais hypogea L. (Abraham and Damodaran, 2012). Plant grown under different levels of cadmium showed a significant reduction in the length of shoots and roots, yellowing and ultra-structural alterations of the leaves and a significant decrease in the essential oil composition (Zheng et al. 2010).

Mercury-

Of all the heavy metals, mercury is found to have significant environmental concern. Once mercury is introduced to the soil, it lasts for a long time because of its indestructible and non-degradable nature and therefore causes potential risk for ecosystems (Leon et.al. 2005) & Alloway (1995). Mercury is not essential for any of the biological functions; rather it is toxic to both plants and animals. In living organisms, mercury is thought to interfere with the mode of enzyme action and protein synthesis by binding with the sulfhydryl groups due to its strong affinity for sulphur. Mercury is known to be the most hazardous heavy metal. Global release of mercury in the atmosphere has been raising three to four folds. However, the anthropogenic origin of mercury at local level is much more. Hg inhibits seed germination not only due to unavailability of sugars to embryo by impairing the solubilization of starch but may also be due to damage caused to embryos produced by Hg treatment (Gautam et.al., 2010).

Cajanus cajan L. (Pigeon pea) is a multiuse legume crop in the world, especially in Indian subcontinent. Due to its multiple uses, pigeon pea is widely grown as inter crop in semi-arid regions. It provides the main source of protein for many poorest population and plays important role in reducing malnutrition for millions of people in the world. Pigeon pea is an ideal source of protein and vitamins in human diet, especially in the vegetarian population. The present study is to assess the effect of different concentrations of mercuric chloride and cadmium chloride on seed germination and seedling growth of *Cajanus cajan L*. (Pigeon pea).

MATERIALS AND METHODS:-

A pulse crop, *Cajanus Cajan, (L)*. (Pigeon pea) was selected for the present study. Pure line uncontaminated seeds of Pigeon pea were obtained from the seed market of Nawegaon, Chhindwara district (M.P.). Seed treatment was not done by any seed dressing chemical and by any antifungal chemicals to avoid any interference during the experiments. Healthy seeds were hand shorted and selected for the experiments.

Test Chemicals: Mercuric chloride ($HgCl_2$) and cadmium chloride (manohydrate) $CdCl_2$, H_2O) were used as test chemicals. Different concentrations of the compounds were prepared using distilled water as solvent.

Five concentrations of the metals (1,5,10,20,50 mg/L) were prepared using distilled water and for control only distilled water was used. Healthy pigeon pea seeds of uniform size were selected and soaked in different concentrations of mercuric chloride and cadmium chloride solution for 24 hours. Twenty seeds were placed at equidistant in each sterilised petri dish lined with filter paper. The filter paper was moistened with 20 ml of each concentration regularly and for control 20 ml of distilled water was used. Three replicates of each concentration were taken for study.

The seeds were allowed to germinate in Remi Seed Germinator (R-6C) with relative humidity 90% and 20°C temperature with 12 hours exposure to tube light which was set in auto mode.

The percentage of germination was studied after 48 hrs. and growth of seedling was evaluated after 10 days treatment. Various growth parameters like root length, shoot length, fresh weight of root and shoot, dry weight of root and shoot were measured in triplicates and mean value was calculated. The 10 days old pigeon pea seedlings were taken for recording fresh weight of shoot and root. They were kept in a hot air oven at 80°C for 48 hours, dry weight of shoot and root were recorded.

Table -1 Seed Germination and Seedling Growth of *Cajanus Cajan* in different Concentrations of $HgCl_2$.

Treatment	Germination percentage	Root Length in cm	Shoot length in cm	Root/shoot Ratio	Fresh weight of Root in gm.	Dry weight of Root in gm.	Fresh weight of shoot in gm.	Dry weight of shoot in gm.
Control	100	6.30	9.20	0.685	0.564	0.052	1.060	0.179
1mg/l	100	6.28 (-0.75)	9.15 (-0.52)	0.682 (-0.26)	0.270 (-50.25)	0.043 (-17.75)	1.062 (+6.25)	0.192 (+8.25)
5mg/l	90	5.01 (-20.54)	8.15 (-12.15)	0.615 (-9.65)	0.200 (-64.25)	0.038 (-22.50)	1.064 (+0.035)	0.190 (+7.20)
10mg/l	80	4.65 (-28.55)	7.25 (-17.25)	0.60 (-9.75)	0.162 (-70.65)	0.025 (-44.25)	0.758 (-24.75)	0.160 (-8.90)
20mg/l	73	3.20 (-50.75)	7.25 (-20.50)	0.410 (-40.20)	0.130 (-76.75)	0.025 (-48.05)	0.770 (-26.22)	0.155 (-11.52)
50 mg/l	70	2.25 (-62.75)	6.52 (-29.70)	0.350 (-48.25)	0.120 (-78.25)	0.020 (-55.25)	0.640 (-38.25)	0.122 (-30.65)

(The figures in parenthesis are % increase / decrease from the control value)

Table -2 Percent of Germination and Seedling Growth of *Cajanus Cajan* treated with different Concentrations of CdCl2. The data were recorded in 7-days old seedlings. Figures in parenthesis indicate % increase/ decrease from Control value.

Treatment	Germination percentage	Root Length in cm	Shoot length in cm	Root/shoot Ratio	Fresh weight of Root in gm.	Dry weight of Root in gm.	Fresh weight of shoot in gm.	Dry weight of shoot in gm.
Control	96	6.32	9.23	0.684	0.563	0.051	1.062	0.178
1mg/l	90	3.28 (-45.35)	7.15 (-18.25)	0.482 (-34.20)	0.230 (-60.25)	0.040 (-27.85)	0.865 (-18.10)	0.160 (-7.58)
5mg/l	80	2.75 (-52.50)	6.15 (-25.15)	0.425 (-35.25)	0.150 (-72.25)	0.025 (-52.50)	0.560 (-46.90)	0.085 (45.10)
10mg/l	70	1.40 (-76.62)	4.40 (-50.85)	0.310 (-52.45)	0.082 (-85.20)	0.015 (-80.10)	0.400 (-60.55)	0.088 (-48.90)
20mg/l	68	0.56 (-90.25)	2.75 (-68.70)	0.195 (-70.40)	0.021 (-95.10)	0.015 (-92.20)	0.221 (-75.75)	0.035 (-77.52)
50 mg/l	63	0.48 (-90.15)	2.52 (-70.30)	0.185 (-68.65)	NA	NA	NA	NA

N. A.: Data not available due to any development of root and shoot after germination at 50mg /l. $CdCl_2$ concentration.

RESULTS AND DISCUSSION:-

The results of germination percentage, root length, shoot length, fresh weight and dry weight of root and shoot with heavy metal treatment to pigeon pea seeds of different concentrations are presented in Table 1 & 2. The germination percentage was maximum (100%, 96%) in control and it declined with the increase in concentration of mercuric chloride and cadmium chloride respectively. The seeds treated with 50mg/L concentration of Mercuric chloride the % of germination was 70% whereas, it was 60% in case of cadmium chloride treatment. Similarly, the maximum root length and shoot length was 6.30 cm and 9.20 cm respectively in the controlled seedlings which was gradually decreased with the increase in concentration of test chemicals. The maximum fresh weight and dry weight recorded in control plants. There is a gradual decrease in the fresh weight and dry weight with the increase in the concentration of mercuric chloride and cadmium chloride treatment. The visible symptoms of toxicity of mercuric chloride and cadmium chloride treatment was stunting and chlorosis, browning of leaf tip, reduction in growth ,stunting of seedlings and root were the morphological symptoms of mercury toxicity. Brown margin of leaves chlorosis, necrosis, brown stunted roots, reddish veins and petioles, purple stems are the visible symptoms of cadmium toxicity. The inhibition of root growth and development of lateral roots are symptoms of toxicity due to mercury which can be attributed in the inhibition of mitosis, reduced synthesis of cell wall components and changes in photo synthetic activity. Similar observations with $HgCl_2$ treatment to Arachis hyposea seeds were noticed by Damodaran et al., (2012).

Amendments are used to immobilize the contaminants, thereby limiting the bioavailable fraction (Bandara et al. 2016, 2017; Herath et al. 2017; Kumarathilaka and Vithanage 2017). In addition to amendments, there are also internal mechanisms in place, which can limit the interaction of heavy metals in plant metabolism. Phytochelatins and metallothioneins are such proteins that may play vital roles in heavy metal de-toxicity in microorganisms and plants (Shen et al. 2010).

FUTURE PERSPECTIVES-:

Heavy metal release to the environment is pronounced growing environmental problem, leading to detrimental effects at the ecosystem level. When early life cycle stages such as seed germination and seedling establishment are affected by heavy metals, there can be lasting consequences at the individual plant, population and community levels. The ways in which heavy metals can cause toxicities, including in storage food mobilization, photosynthesis and plant osmoregulation, can vary depending on the type of heavy metal, its speciation, soil pH, plant species and the mobility of the metal. In order to overcome heavy metal induced toxicities, plants employ numerous mechanisms, including the activation of the antioxidant system, proline production, production of heat shock proteins and acetyl salicylic acid, among others. There are many informational gaps relating to the study of heavy metal influence on seed germination and seedling establishment. Some of these areas are summarized below to generate interest for further investigation.

Seed germination and early seedling growth are two important stages of plant development. An understanding of the multitude of ways in which heavy metals influence these critical stages in plant growth and development is fundamental for advancing plant science and developing better practices in agriculture, forestry and restoration. This review highlights the primary ways in which heavy metals impact critical stages of plant development in the hope of fostering additional research for improving our knowledge on the deleterious effects heavy metals have on seeds and seedlings and how such effects could be minimized with additional research in phytotechnologies.

CONCLUSION:-

The results obtained in this investigation show that Mercury chloride and Cadmium chloride at higher concentrations show adverse effects on seed germination and seedling growth. Moreover the release of Mercury chloride and Cadmium chloride into the immediate environment may enter the cereals, pulses and vegetables we eat and affect the health of human beings. Hence it is an urgent necessity to minimize the use of Mercury chloride and Cadmium chloride in industries containing pesticides and fungicides. Further work is required to biochemistry and enzymatic level to evaluate the toxic effect of the heavy metals on the physiology of the test plant.

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