ISSN (Online): 2091-2609
DOI Prefix: 10.3126/ijasbt

International Journal of Applied Sciences and Biotechnology (IJASBT)

Research Article

Effect of Mercuric Chloride ($HgCl_2$) Stress on DNA, RNA and Enzymes *Vigna mungo* L. Seedlings

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Article Information

Received: 08 February 2022
Revised version received: 12 March 2022
Accepted: 15 March 2022
Published: 29 March 2022

Cite this article as:
M. Mohapatra (2022) Int. J. Appl. Sci. Biotechnol. Vol 10(1): 16-20. DOI: 10.3126/ijasbt.v10i1.44153

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Peer reviewed under authority of IJASBT

Abstract

At elevated concentrations all heavy metals are potentially toxic. Effects of Mercuric Chloride on the morphological parameters such as DNA, RNA and Enzymes were analysed during investigation on 10th day in seedlings of *Vigna mungo* L., All parameters exhibited reduced trend with increase in $HgCl_2$ concentrations when compared to control plants but the enzyme activities increased. This indicates that, the seedlings are in the process of increase in tolerance to $HgCl_2$ treatment. Metal toxicity affects crop yields, soil biomass and fertility.

Keywords: Mercuric Chloride ($HgCl_2$); Pulse; *Vigna mungo*; DNA; RNA; Enzymes

Introduction

Black gram (*Vigna mungo* L. or P*haseolus mungo* L.) is one of the most important pulse crops for human consumption in India and Middle East. Black Gram (*Vigna mungo* L.*)* is one of the most widely available pulse crops of India. Reference of black gram is found in ancient Indian literature of Kautilya and Charak. In those days seeds of black gram have been recovered from chalcolithic site, Navdatoli-Maheswar of India dated back to 1600-1400 BC. It is now cultivated in tropical subtropical regions of the world including India, Malaysia, East Africa and many southern European countries of the world.

The green pods are rich in protein, calcium, vitamins, magnesium, sodium, iron and fibres which are highly nutritious, it plays a vital role in improving the soil health through biological nitrogen fixation. Their yield is low and unstable due to water and other stresses during growth stage. Heavy metals are integral components of ecosystems. The distinctiveness characteristics of heavy metals are

poisoning and results in the alteration in different biochemical parameters and inactivation of enzyme systems.

All heavy metals are potentially toxic at elevated concentrations. Effects of Mercuric Chloride on the morphological parameters as well as different biochemical parameters were analysed during investigation on 10-day old seedlings. All parameters exhibited reduced trend with increase in $HgCl_2$ concentrations when compared to control plants but the enzyme activities of Catalase. Peroxidase and Polyphenol Oxidase increased. Because heavy metals cannot be degraded or destroyed, they accumulate in the human body and are not broken down, concentrating in the liver, kidneys, brain, skeleton, and keratinised tissue such as hair and nails.

The most common heavy metal contaminants are Lead (Pb), Cadmium (Cd), Mercury (Hg), Chromium (Cr), Copper (CU), Nickel (Ni), and Zinc (Zn), All heavy metals are potentially toxic at elevated concentrations. The uptake of overload concentrations of heavy metals reduces the plant growth. This alteration in the plant growth is correlated with disruptions of physiological and cytological processes in plant cells and by this way the process of respiration, photosynthesis and mitotic activities are greatly affected by toxic effects of heavy metals.

Mercury and Its Sources

Mercury is a typical toxic trace metal pollutant. Bioaccumulation of Hg in plants and its entry into the food chain resulting in long term health hazard is of major concern.

Mercury (Hg) is a global environmental pollutant that is present in soil, water, air and biota. Mercury enters the environment as a result of both natural as well as manmade activities.

Combustion of fossil fuels, wood, industrial wastes and effluents, sewage sludge and crematories, mining, (Mehra and Farago, 1994), high temperature processes, e.g. smelting, cement and lime production are the major anthropogenic sources of Hg.

The other causes of mercury pollution includes manufacturing/commercial activities: e.g. metal processing, gold extraction, Hg mining, chlor-alkali plants, chemical and instrument industry (Hg chemicals, paints, batteries, thermometers, process reactants and catalysts), agriculture application (pesticides, fertilisers and manure) (Foy *et al*, 1978), landfills, seed dressing (fungicides), incineration of coal, other sources like cosmetics, dental fillings, preservatives, medical and laboratory wastes.

Present investigation is aimed to study Mercuric Chloride action on Nucleic acids (DNA and RNA) and enzyme activities (Peroxidase, Polyphenol Oxidase and Catalase) of Black Gram (*Vigna mungo L.*

Materials and Methods

The Plant Material

Vigna mungo L. which belongs to Kingdom- Plantae, Order- Fabales, Family- Fabaceae, Genus- *Vigna*, Species- mungo L., Common name: Urad Dal, Variety: PU31(Urad Bean) (Fig.1).

Selection of Plant Material

The test plant for the present study is *Vigna mungo L.*(Black gram).

The seeds *of Vigna mungo L.*were collected from OUAT, Ankushpur, Odisha. Healthy seeds of uniform sizes were used. Pure line *Vigna mungo* L. is procured from the local agricultural body and used for investigation. These certified seeds were examined under preliminary selection for uniformity of size, shape and colour and then healthy seeds were sorted out by hand, sieving and sorting before experiment.

Fig. 1: Plant of Black gram (*Vigna mungo* L.) with pods.

Selection of Effective Concentration

The metal exerts both promoting and retarding effect on the germination and seeding growth of the plant and it was essential to select a limited no. of concentration for further experiments. Thus, five concentrations of $HgCl_2$ (5, 15, 25, 35, & 45mg/l) were selected on the basis of LC 50 test. The data obtained from the germination and seedling growth experiments were used for screening.

Parameters Evaluated

The seedling parameters studied were DNA, RNA (Schenider,1957) and enzymes (catalase, Peroxidase and polyphenol Oxidase by Kar and Mishra (1976)) of the 10 days old seedlings after treatment with the stress chemicals following standard procedures

Results and Discussions

The results obtained in the present experiment was given in the form of graphs in Fig 2-5. The Changes in DNA and RNA content were given in Fig 2 and 3. The maximum DNA content in shoot was found in control and amounts of DNA decreased up to 45mg/l. For RNA there was a rise at 5mg/l concentration and after this there was a decrease in the contents up to 45mg/l concentration. In root the amount of DNA went on decreasing gradually with increase in Mercuric chloride concentration i.e. control had the maximum content and 45mg/l concentration had least content of DNA. Maximum amount of RNA was found in the concentration 5mg/l while least amount was found in 45mg/l. The enzyme activity like POD and Polyphenol oxidase in shoot increased with the increase in mercury dose, however, the catalase responded less prominently. In case Root of the seedlings, the enzymes like Catalase and Peroxidase did not respond to the mercury treatment whereas Polyphenol oxidase showed an increasing trend (Fig 4 and 5).

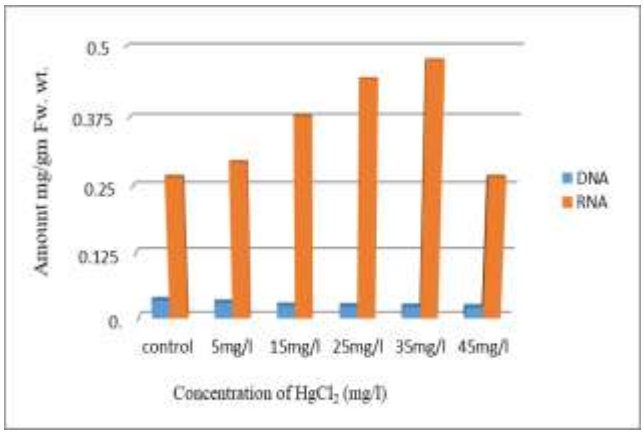

Fig. 2: Effect of Mercury chloride stress on contents of nucleic acid (DNA & RNA) in Shoot of 10 days old seedlings of *Vigna mungo*, L.

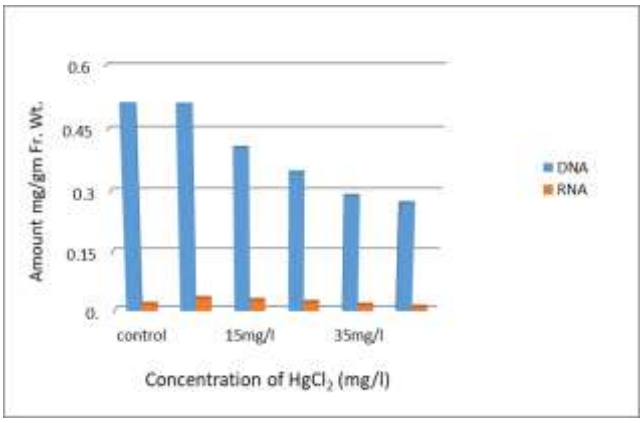

Fig. 3: Effect of Mercury chloride stress on contents of nucleic acid (DNA & RNA) in root of 10 days old seedlings of *Vigna mungo*, L.

The enzyme activities (catalase, peroxidase and Polyphenol oxidase (PPO) of shoot and root were studied by following the procedures of Kar and Mishra, (1976). The Catalase enzyme activity was expressed in terms of ůmol H_2O_2 released $min^{-1}g^{-1}$ fresh weight., POD (Peroxidase) activity is in absorbancy units (A_{420} nm) and PPO activity is in absorbancy units (A_{420} nm).

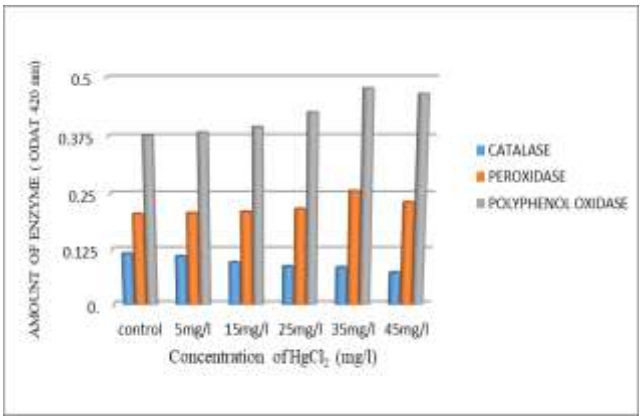

Fig. 4: Enzyme content (catalase, Peroxidase and Polyphenol oxidase) of Shoot of the 10days old seedlings of *Vigna mungo* L. (root) with different concentrations of $HgCl_2$.

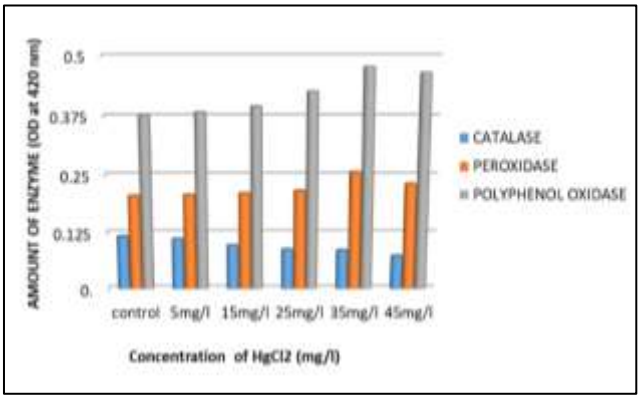

Fig. 5: Enzyme content (catalase, Peroxidase and Polyphenol oxidase) of root of the 10days old seedlings of *Vigna mungo* L. (root) with different concentrations of $HgCl_2$.

In both roots and shoots the enzyme activities increased with increase in different concentrations of $HgCl_2$.

Mercury has always been reported to be more toxic compared to other heavy metals like cadmium (Nordberg, 1976; Rai *et al*., 1981; Fergusson, 1990; Kneer and Zenk, 1992; Gadallah, 1994; Shaw, 1995). Chromium (Garg *et al*., 1989) and lead (Jindal and Kaur, 2000; Orcutrt and Nilsen, 2000)

Essential elements at high concentrations and non- essential elements even at low concentrations are toxic (Baker and walker;1989), Mehera and Farrago, (1994). Metals in the environment operate as stress factors causing physiological changes (strain) and in doing so reduce vigour, or in the extreme case cause death (Levitt, 1980).

The DNA contents decreased but RNA contents increased in some increasing concentrations of $HgCl_2$ in our study which is further strengthened by the following observations. Duan *et al*. (1992) observed an increase followed by decreasing trend in *Vicia faba i*n the content of RNA in response to Cadmium. Angadi *et al*. (1996) observed that increase in RNA in response to cadmium and there was a decrease at higher concentration of the metal. However, De Filipps and Pallaghy (1976) reported contradictory findings in *Chlorella.*

Heavy metals reduce the soluble protein in agricultural crops (Hemlatha *et al*., 1997) as observed in this study too. The decrease is caused either by a reduced de novo synthesis or by an increased decomposition of proteins to amino acids. This observation was also confirmed in our study though further research in this regard is necessary for confirmation.

Vallee and Ulmer (1972), have emphasised that heavy metal ions have strong affinities for side chain ligands of proteins, indicating that enzyme activities and other functions are affected by heavy metal ions. A reduction in the amount of proteins was observed in seedlings of *Zea mays* at all concentrations of mercury employed by Kalimuthu and Sivasubramanian (1990). Similarly, a concentration dependent decrease in soluble protein content over the control was observed in the leaves of *Albizia lebbek* (Tripathi and Tripathi, 1999).

Proline content, electrolyte leakage, and malonyl aldehyde content (shoots and roots) were significantly lower in inoculated plants with respect to un inoculated plants under mercury stress. Therefore, it could be assumed that all these parameters collectively improve plant growth under mercury stress conditions in the presence of PGPR. Hence, these PGPRs can serve as promising candidates for increasing plant growth and also have immense potential for bioremediation of mercury-contaminated soils.

Hg is a critical pollutant which can affect many ecosystems with toxic effects on many biological processes. Plants play fundamental role as the base of many trophy chains and they help in subsistence of mankind and humanity. It is therefore essential to enhance the understanding the mechanism of Hg uptake by plants and the various metabolic process that are targeted by this pollutant. This work could be a valuable source for other investigators to extend their research in this area of research.

Conflict of Interest

The authors declare that there is no conflict of interest with present publication.

Acknowledgements

Author is thankful to Principal, Khallikote Autonomous College, Berhampur, Odisha for encouragement and to carry out this project work in the department.

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