International Journal of Scientific Research in Biological Sciences **Research Paper**
Vol.7, Issue.5, pp.135-139, October (2020) **E-ISSN:** 2347-7520

Study on the Seeds Germination and Biochemical Parameters (Chlorophyll, Proline, and Ascorbic Acid) of Soybean & Chickpea under the Influence of Heavy Metal (CD) Stress

Ruhi Khan[1]*, Gulafshan[2], Farha Rehman[3], Aabshar Khan[4]

[1,2,3]Department Of Botany, Mohammad Ali Jauhar University, Rampur (U.P.) India
[4]Department Of Biochemistry, Mohammad Ali Jauhar University, Rampur (U.P.) India

**Corresponding author: khanruhi865@gmail.com, Tel: +91 7668870136*

Available online at: www.isroset.org

Received: 28/Sept/2020, Accepted: 09/Oct/2020, Online: 31/Oct/2020

Abstract- In the world population, the industrial & environmental pollution, and chemical toxicity raising. High speed industrialization and urbanization processes has show to the synthesis of pollutants such as pesticides, salt , petroleum products, acids and heavy metals in the natural resources. These pollutants affects both plants and animals. Heavy metals as well as lead, nickel, cadmium, copper, and mercury are important environmental factors that cause toxic effects on plants and animals. Here , we are study about Cadmium (Cd). It is possess dangerous threats on the agro-ecosystems. Cd act as a poisonous effect on the physiology of plants. In this review, we have summarized the effects of different concentrations (0, 100 ppm, 200 ppm ,and 400ppm) of heavy metal (Cd) on germination process of seeds and biochemical parameters i.e., (chlorophyll, proline, and ascorbic acid) of soybean & chickpea. In our experiment the data revealed that higher concentration (200ppm) of Cd increase the levels of proline and ascorbic acid, but decrease the chlorophyll content in leaf of soybean & chickpea plant. While this report exist on , heavy metal (Cd) act as stress for plants life , and how plants responses to overcome. The future scope of this work remains in dig out the signaling mechanisms in germinating seeds in response to any heavy metal stress.

Keywords: Cadmium, germination, heavy metals, soybean, stress, chickpea & proline.

I. INTRODUCTION

Valuable and non-renewable resource which essential for germination of seeds is soil, However, in the contemporary world, countless soil pollutants restrict the growth of plants. Abiotic stress as the negative impact on non-living factors & living organisms in a specific environment. The stress include extreme temperatures, salinity, drought, chemical toxicity and oxidative stress , that cause major worldwide crop loss that pose serious threats to agricultural purpose. With the continuing advancements in urbanization and industrialization process, releases of toxic contaminants like heavy metals in the natural resources . Metal toxicity has become a serious problem of worldwide, and which affects crop yields, soil biomass and soil fertility. All life form on the earth possess harmful health consequences with the presence of heavy metals, like nickel, cobalt, cadmium, copper, lead, chromium and mercury in air, soil and water, and affecting the entire ecosystem. The major sources of pollution in the state of Odisha in India are an excessive burdens of mine, industrial effluent, fertilizers and pesticides, high salts, heavy metals, and elements that degrade the soil quality **(1)**. In this study, the Cd and others chemicals at higher concentration hamper the plant germination, growth and production mainly associated with the physiological, biochemical and genetic elements of the plant system, and reduce the crop yield. Effect of heavy metals on seeds germination & plant biochemical parameters, and is also reported to be toxic to most plant species affecting amylase, protease and ribonuclease enzyme activity **(2).** It has been also reported to affect the mobilization of food reserves like proteins , lipids, and carbohydrates in the process of seeds germinating **(3).** Heavy metal stress has been describes to affect photosynthetic pigments, yield and cause accumulation of Na^+, K^+ and Ca^{2+} in mung bean **(4).** Cadmium (Cd) has been exhibit to give rise to delay in germination, induce membrane damage, and reserve food mobilization by increased cotyledon/embryo with the ratios of total soluble sugars, glucose, fructose and amino acids **(5).** The union of Ni and NaCl in seeds germination of Brassica nigra causes remarkable decline in plant growth, leaf water potential, pigments and photosynthetic machinery by increasing electrolyte leakage, lipid peroxidation, H2O2 content, activity of anti-oxidative enzymes , and the level of proline content **(6).** Contamination of heavy metals in agricultural soil ,and in all around industrial areas is a serious problem. Such impurities is largely due to injudicious anthropogenic activities like use of pesticides , salinity ,and containing heavy metals in agriculture. Carry out of untreated industrial wastes ,and high rate of burning of fossil fuels, mining etc.**(7).**Generally, effects of the Cd of the water balance of plants by reducing root growth, limiting water uptake which reduction in vessel size, and causing partial stomatal closure **(8).** Cd basically enters the roots of the plants by the mechanism of diffusion.The depletion of biomass in the presence of Cd toxicity could be the direct effect of the inhibition of chlorophyll synthesis in chickpea (*Cicer arietinum* L..) **(9)** ,and photosynthesis in tomato **(10).**

II. MATERIALS & METHODS

Our Laboratory experiments perform with *Glycine max* (soybean) and *Cicer arietinum* (chickpea) seeds , in the Department of Botany, S.S. Faculty Of Science, Mohammad Ali Jauhar University Rampur (U.P.), India. Healthy seeds of C. arietinum were collected from farm of Agriculture, and seeds of Glycine max collected from authentic seeds source of Rampur. These seeds were kept in air-tight clean packets at normal room temperature and was use as a experimental materials. After collection of seeds , then surface was sterilized in 0.1% $HgCl_2$ solution for 20 seconds. In this experiment was taken 24 petridishes i.e., 12 petridishes used in soybean seeds germination , and other 12 petridishes used in chickpea seeds germination. The process of both experiment are the same, here we are identify the responses of heavy metal (Cd) on germination percentage of both seeds at same time (15 days). The process of seeds germination follow the **(11).** The temperature of this experiment setup was kept at 22°C, with one hour exposure to sunlight. The experiment was carried out to the randomized block design with three replicates in vitro-conditions. Ten seeds with cotton was put in each petridishes , and apply the solution of Cd with different doses (0, 100ppm, 200ppm, and 400ppm) on all petridishes of soybean & chickpea except control. Responses of both seeds under Cd stress was write down every day , and experiment was finish on 15 days. After this experiment , the germinated seeds was sown in pot (12cm) with soil (kg^{-1}), for the estimation of biochemical parameters. When the plant growth occur , collect the leaf of both plants (soybean & chickpea).In our next experiment describes the effect of Cd on biochemical parameters i.e., chlorophyll, proline , and ascorbic acid. The estimation of total chlorophyll was perform of the matured leaf by spectrophotometer using the method of **(12).** Proline (Pro) content was measured using the ninhydrin reagent and toluene extraction **(13).** Non-enzymatic antioxidants such as ascorbic acid (ASA) concentration was define by using a slightly modified method of **(14).**

Statistical Analysis

The estimated data were analysed statistically by one-way, and two-way ANOVA, for a completely randomized design as described by **(15),** and the data represented the average of the three replicates.

III. RESULTS

Cadmium stress

Heavy metal (Cd) stress has been long & major environmental pollution, and threatends in all living forms of life. Cadmium is one of the highly toxic worldwide environmental factor that cause serious problems in agriculture. Bioaccumulation of large concentrations of Cd in plants enter the food chain , and affects on the both animals & humans.

Germination of both seeds (*Glycine max* and *Cicer arietinum*) under Cd stress

The percentage of germination decline with increasing levels (0, 100ppm, 200ppm, and 400ppm) of Cd. Heavy metal (Cd) possess negative impact on germination of both seeds of soybean & chickpea. In our both experiments the highest percentage (95% & 90%) of germination value was observed at control. In fig. a show all percentages of germination with increasing level of Cd. At low level (100ppm) of cadmium concentration germination percentage (80%&85%) was not inhibit as compared with untreated seeds. At the medium level of Cd (200ppm) notify are rare number of percentage seeds germination(65%& 60%).Over-all result describe that the maximum level (400ppm) of heavy metal (Cd) completely reduce the seeds germination (45%&40%) process in vitro condition at 15 days .

Biochemical parameters under the influence of Cd stress

Chlorophyll

As in fig. b chlorophyll contents of *Glycine max.* & *C. arietinum* reduce due to heavy metal (Cd) stress. Generally the total chlorophyll content decline with raising concentration of Cd (0, 100ppm, 200ppm, and 400ppm). At maximum level of Cd (400ppm) chlorophyll superior decline with other concentrations (100ppm, & 200ppm) of heavy metal (Cd) and control.

Proline

Maximum proline accumulation of *Glycine max.* & *C. arietinum* was observed with 400ppm Cd level as compared with control experiment (fig. c). Heavy metal stress show positive impact against proline contents, as Cd stress concentrations increase proline levels also increase in plants. Low proline accumulation was observed at 100ppm as compared with 200ppm & 400ppm treatment of cadmium.

Ascorbic acid

In our experiment, in the leaves of soybean & chickpea perform the estimation of ascorbic acid at different Cd concentrations i.e., (0,100ppm, 200ppm,and 400pp). It occurs in all plant tissues, usually being higher in photosynthetic cells and meristems (and some fruits). In our work, ascorbic acid also raising with increasing Cd treatment levels. Low content of ascorbic acid write down at control as compared with all Cd treatment concentrations. The medium level of ascorbic acid was observed on the 200ppm, and large amount of ascorbic acid was found at the 400ppm.

IV. DISCUSSION

In the present study, significant reduction in germination percentage at different Cd concentrations as contrast to control experiment **(16).** This result are correlated with the result of different crops species reported by various researcher in different plants species **(17).** Various abiotic stresses decline the chlorophyll content in plants **(18).** Our result on decrease in chlorophyll content with increasing heavy metal stress levels , corroborated with the result of **(19).** Proline also acts as a major reservoir of energy and nitrogen fixation, which can be used in resuming the growth of the plant **(20)** after the stress removal. Proline accumulation, in shoots of *Brassica juncea, Triticum aestivum* in response to cadmium toxicity was demonstrated by **(21)** ,but they found that proline accumulation decreased with the exposure to cadmium in hydrophytes.Under physiological conditions , ascorbic acid act as a reducing agent . Ascorbic acid is the most powerful and water soluble antioxidant , and acts to prevent in the damage caused by ROS in plants **(22).**.

V. CONCLUSION

In our results indicate the impact of different concentrations of Cd on seeds germination in vitro condition, and an increase pigment content, proline, & ascorbic acid due raising concentration of Cd treartment. There are not appear significant different between in the experiment of Glycine max (soybean) and Cicer arietinum (chickpea) . Our result suggested that contamination of HMs in soil and water , changing environment ,possess a serious threat to public health and food safety, and major hazard to animals & plants.

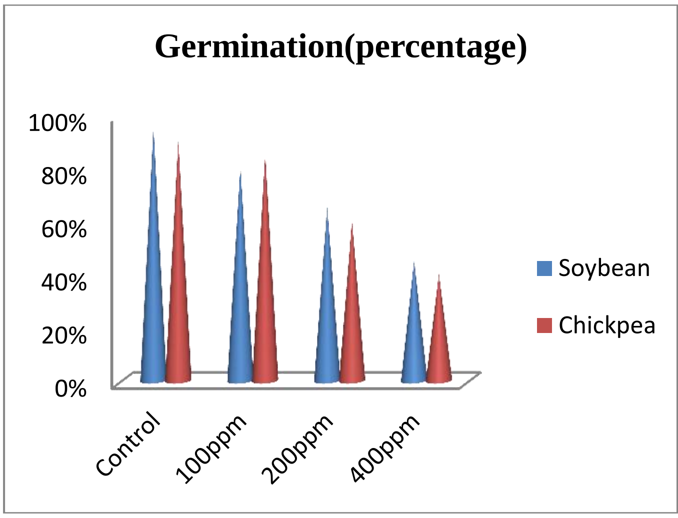


Figure. a

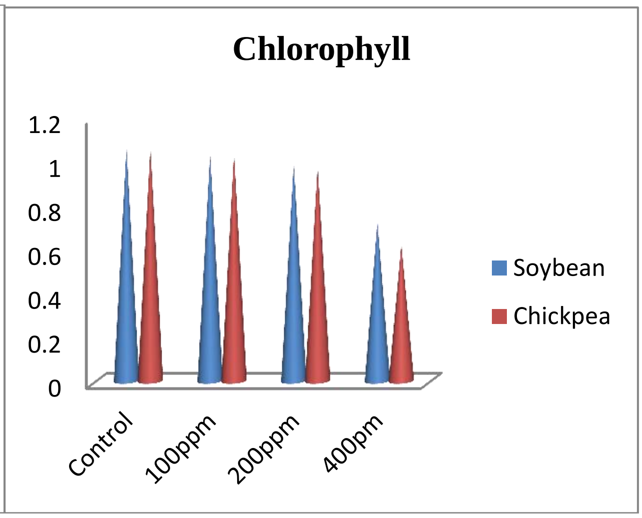


Figure. b

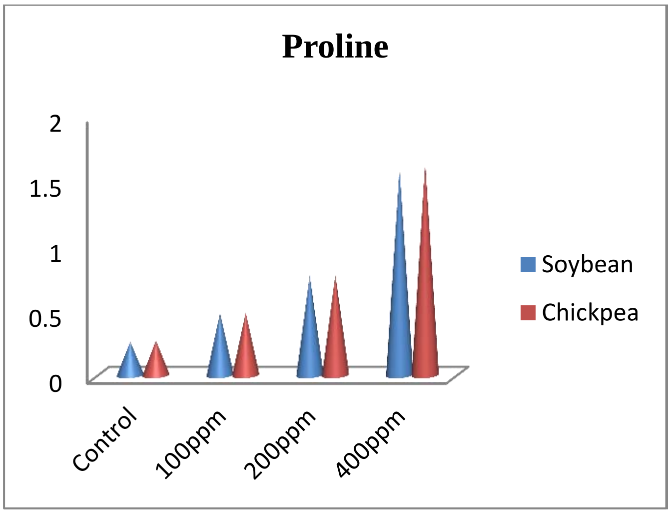


Figure. c

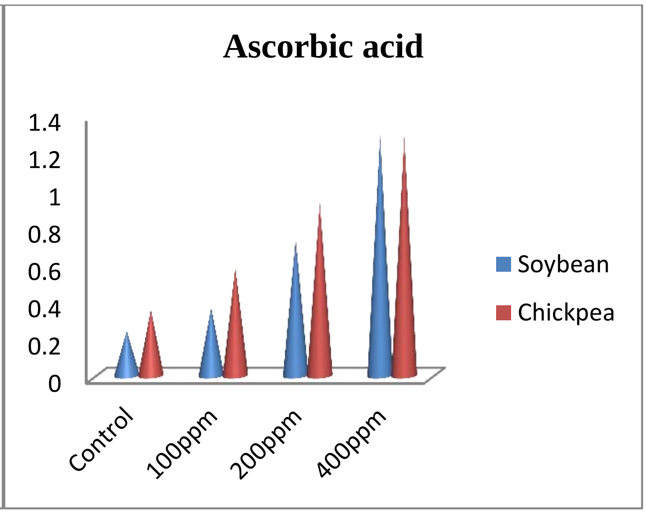
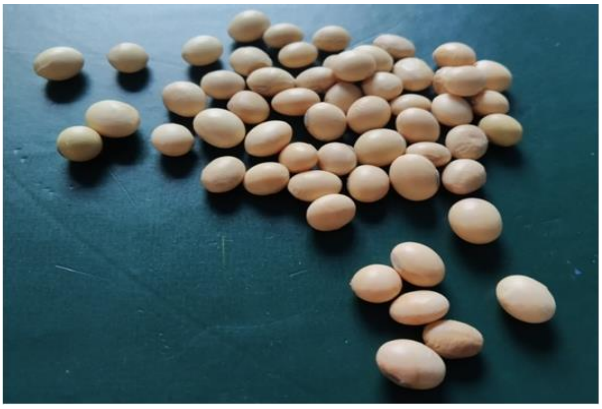


Figure. d

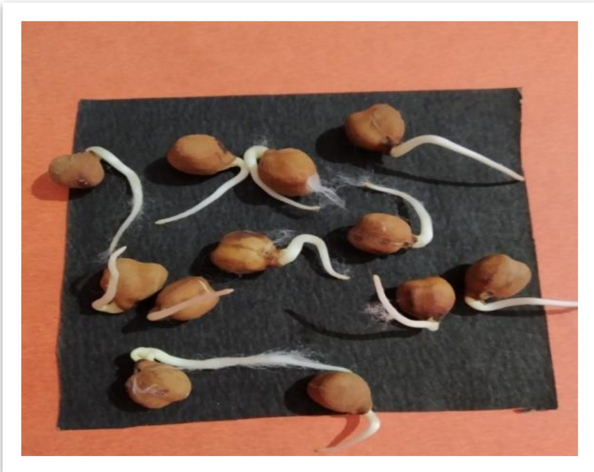
Effect of different concentrations (0, 100ppm, 200ppm, & 400ppm) of Cd on seeds germination (%), and biochemical parameters i.e.,(Chlorophyll, Proline, & Ascorbic acid).



(A) Raw seeds of soybean (*Glycine max*)

(B) Raw seeds of chickpea (*C. arietinum)*

(C) Germinated seeds of soybean (*Glycine max)*



(D) Germinated seeds of chickpea (C. *arietinum)*

(E) Under growth condition soybean (*Glycine max)*

(F) Growth condition of chickpea (*C. arietinum)*

ACKNOWLEDGMENT

Author are thanks to assistant professors , Department of Botany, Mohammad Ali Jauhar University, Rampur India. In our work overall support by them , I also thanks to Chancellor sir Mohd. Azam Khan , Mohammad Ali Jauhar University, Rampur, for providing facilities in this work

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