International Journal of Research in Biosciences
Vol. 3 Issue 1, pp. (19-24), Jan 2014
Available online at http://www.ijrbs.in
ISSN 2319-2844

Research Paper

Effect of the stress induced by mercury and cadmium on the biochemical parameters of the seedlings of pigeon pea (*Cajanus cajan* (L.) *Millsp.)*

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(Received November 23, 2013, Accepted December 31, 2013)

Abstract

The effect of Mercuric chloride and Cadmium chloride on the biochemical parameters of the seedlings of Pigeon pea (*Cajanus cajan (L.) Millsp)* were studied. The seeds were germinated in Petri plates in different concentration (0, 1, 5, 10, 20 mg^{-1}) of mercuric chloride and cadmium chloride for 10 days in laboratory conditions. The biochemical parameters like photosynthetic pigments, sugar, protein, amino acid, DNA and RNA contents of 10 days old seedling were analysed. There was a gradual decrease in photosynthetic pigments and biochemical constituents of the seedlings with the increase of metal concentrations. A significant negative correlation was found between the metal concentrations and the biochemical constituents.

Keywords: Mercury, cadmium, pigeon pea, biochemical content.

Introduction

Pigeon pea is an important source of protein. It is a major source of 'dal' and important constituent in the food habit of Indian people. Pigeon pea crop improves soil fertility status ensuring better growth of succeeding crop. Production of the crop has significantly increased in the state of Maharashtra, Gujarat, Andhra Pradesh, Odisha and Tamil Nadu. Despite the growth rate in productivity, it has not been possible to meet the demand of ever growing population.

The degradation of environment due to industrialisation has been at an alarming rate. Most of the industrial wastes releases to environment contain varieties of heavy metals. Heavy metals cause pollution after reaching certain concentration in soil. Mercury and cadmium has no essential function but cause toxicity above certain tolerance level. Heavy metals are highly toxic to plants. Their uptake and accumulation by plant tissues cause various morphological, physiological and biochemical response[1].

Heavy metal stress causes direct and indirect effects on all physiological processes of plant [2] Agricultural use of mercuric chloride containing fungicides such as mancozeb (16% mn and 2% Hg), Zineb and Ziram (1-18% Hg) may yet another source of mercury in the environment, [3,4]. Authors reported that at high concentration of $HgCl_2$, a significant decline in chlorophyll content was occurred in Mulberry plant, which in turn led to depletion in primary production and total production.

Some metals, such as Zinc (Zn), Mercury (Hg), copper (Cu), arsenic (As), Lead (Pb) and cadmium (Cd) may be introduced into the environment by many anthropogenic activities, such as mining, fertiliser use, metal based pesticides and a wide range of industrial activities which release metals into the environment[5] .Cadmium is a toxic metal because of its relatively high mobility in the soil-plant system[6]. Cadmium can induce severe disturbances in physiological processes of a plant, such as photosynthesis, water relations and mineral uptake[7],[8] .Cadmium is easily translocated from plant roots to above ground tissues[9] .Cadmium stress leads to protein degradation through amino acid metabolism resulting in decreased plant growth.

The pulse crop pigeon pea has a high commercial and nutrient value. The objectives of the present study is to find out the variation of chlorophyll, carotenoid , sugar, protein, amino acid, DNA and RNA content of the pigeon pea seedlings in response to the stress induced by Mercury and Cadmium.

Materials and Method

Test material

Seeds of Pigeon pea (*Cajanus cajan (L.) Mill sp.)* was collected from CPR, OUAT, Ratnapur with label UPAS-120 for study.

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

Four concentration of the test chemical (1, 5, 10, 20 mgl^{-1}) 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 Mercury Chloride and Cadmium Chloride solutions for 24 hours. Twenty seeds were placed at equidistance in each sterilised petri plates lined with filter paper. The filter paper was moistened with 20 ml of each concentration regularly. Three replicates of each concentration were taken for the study.

The seeds were allowed to germinate in Remi Seed germinator (R- 6c) with relative humidity 90% and 20^{o}C temperature with 12 hours exposure to tube light which was in auto mode.

The shoot and root of 10 days old seedling were taken for investigation. The biochemical parameters like total chlorophyll[10] Carotenoid [11] , sugar[12], proteins[13,]), amino acid [14], DNA and RNA[15] . content of the pigeon pea seedlings were estimated and analysed by following the standard procedure.

Results and Discussion

The results were presented in Table-1. Total chlorophyll content decreased with increase in the concentrations (1, 5, 10, 20 mgl^{-1}) of $HgCl_2$ and $CdCl_2$ treatment. In 20 mgl^{-1} concentration of $HgCl_2$ and $CdCl_2$ the decrease in total chlorophyll content was 36.20% and 68.96% over the control value respectively.The sugar content of shoot and root of untreated (control) seedlings were 6.17 and 5.82 mg g^{-1} fresh weight. The sugar content decreased with increase in the concentrations of heavy metals, compared to control. A decreasing trend in protein content of shoot and root with increasing concentrations of $HgCl_2$ and $CdCl_2$ (1, 5, 10, 20 $mgl^{-1)}$ was shown in (Table 1).

The maximum decrease was 30.90% in shoot and 61.53% in root over the control values in 20 mgl^{-1} $HgCl_2$ treatment. A significant positive correlation was observed between the concentrations of heavy metals and the amino acid content of the pigeon pea seedlings. A reduction in DNA and RNA content was observed in shoots and roots of the seedlings with increased $HgCl_2$ and $CdCl_2$ (1, 5, 10, 20 mgl^{-1}) application.Mercury and Cadmium cause a decrease in chlorophyll, sugar, protein, DNA and RNA content of pigeon pea seedlings. Decrease in chlorophyll is the primary bioindicator of mercury and cadmium phytotoxicity.

Table 1: Variation in the Pigment Content and Biochemical Parameters of Pigeon Pea Seedlings Treated With Mercuric Chloride and Cadmium Chloride

Treatment	Metal conc.	Total chlorophyll shoot	Carotenoid mg g^{-1} shoot	Sugar Content mg g^{-1} fr.wt.		Protein content mg g^{-1} fr.wt.		Amino Acid content mg g^{-1} fr.wt.		DNA content mg g^{-1} fr.wt.		RNA content mg g^{-1} fr.wt.	
		Shoot	Shoot	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Mercuric Chloride ($HgCl_2$)	Control	0.58	0.014	6.17	5.82	55.01	52.01	5.43	4.34	0.82	0.78	3.5	3.2
	1 mgl^{-1}	0.55 (-5.17)	0.014 (0.0)	6.10 (-1.13)	5.60 (-3.78)	52.0 (-5.45)	50.0 (-3.84)	5.89 (+8.47)	4.70 (+8.29)	0.85 (+3.16)	0.83 (+6.41)	3.7 (+5.71)	3.5 (+9.37)
	5 mgl^{-1}	0.51 (-12.06)	0.013 (-7.14)	5.92 (-4.05)	5.35 (-8.07)	50.02 (-9.09)	35.03 (-32.69)	6.27 (+15.46)	4.98 (14.74)	0.92 (*+12.19)	0.85 (+8.97)	3.9 (+11.42)	3.0 (-6.25)
	10 mgl^{-1}	0.45 (-22.41)	0.013 (-7.14)	5.65 (-8.43)	5.10 (-12.37)	45.02 (018.18)	31.01 (-40.38)	6.58 (+21.1)	5.30 (+22.11)	0.80 (-2.43)	0.76 (-2.56)	2.7 (-22.85)	2.7 (-15.62)
	20 mgl^{-1}	0.37 (-36.20)	0.012 (-14.28)	5.12 (-17.01)	4.76 (-18.21)	38.01 (-30.90)	20.0 (-61.53)	6.72 (+23.75)	5.48 (+26.26)	0.65 (-20.73)	0.65 (-16.66)	1.2 (-65.71)	2.0 (-36.5)
	Correlation coefficient 'r' value	-0.990***	-0.928**	-0.999***	-0.979***	-0.966**	-0.954**	+0.881*	+0.907*	-0.828*	-0.832*	-0.880*	-0.972**
Cadmium Chloride ($CdCl_2$)	Control	0.58	0.014	6.17	5.82	55.01	52.01	5.43	4.34	0.82	0.78	3.5	3.2
	1 mgl^{-1}	0.55 (-5.17)	0.013 (-7.14)	5.82 (-5.67)	5.56 (-4.46)	50.50 (-8.18)	41.50 (-20.19)	5.78 (+6.62)	4.68 (+7.83)	0.81 (-1.20)	0.81 (+3.84)	3.1 (-11.42)	3.2 (0)
	5 mgl^{-1}	0.50 (-13.79)	0.013 (-7.14)	5.44 (-11.83)	5.10 (-12.37)	48.9 (-11.09)	38.70 (-26.57)	5.95 (+9.57)	4.92 (+13.36)	0.85 ((+3.65)	0.80 (+2.43)	3.0 (-14.28)	3.0 (-6.25)
	10 mgl^{-1}	0.37 (-36.20)	0.011 (-21.42)	4.62 (-25.12)	4.37 (-24.91)	41.20 (-25.09)	31.40 (-39.61)	6.12 (+12.70)	5.08 (17.05)	0.78 (-4.8)	0.76 (-2.56)	2.5 (-28.57)	2.7 (15.62)
	20 mgl^{-1}	0.18 (-68.96)	0.009 (-35.71)	3.71 (-39.87)	3.92 (-32.64)	28.6 (-48.0)	18.20 (-65.0)	6.20 (+14.18)	5.32 (+22.58)	0.52 (-36.50)	0.49 (-37.17)	1.8 (-48.57)	1.2 (-62.50)
	Correlation coefficient 'r' value	-0.997***	-0.980***	-0.992***	-0.970***	-0.991***	-0.965***	+0.853**	+0.921**	-0.905***	-0.910*	-0.983***	-0.951**

The values in parenthesis represent the percent increase / decrease over the control value., * Significant at p≤ 0.05, ** Significant at p≤ 0.01, ***Significant at p≤ 0.001, NS: Not significant.

It has been assumed that impaired chlorophyll development by heavy metals may be due to the interference with synthesis of proteins, the structural components of chloroplast[16]. Cadmium has been found to show an adverse effect on chlorophyll content in triticum aestivum[17],[18]. Investigation[19] showed that increased concentration of cadmium caused significant loss of chlorophyll pigment in hydrilla plant. A decreased pigment content with increase in level of Mercuric chloride treatment in green gram (*vigna radiata* L.)[20] The heavy metal cadmium caused a diminished carbohydrate concentration in sugar beet (*Beta vulgaris*)[21]. Protein content was minimum in *Phaseolus vulgaris* L. plants at highest concentration of lead (Pb) and cadmium (Cd)[22] There was a negative correlation between the increase in metal concentration and the decrease in protein levels in Hydrilla plant in response to mercury and cadmium[19] .

In the present investigation Amino Acid content increased with an increase in the concentration of Mercuric Chloride and Cadmium Chloride in both shoots and roots of pigeon pea seedlings. An increase in amino acid content in Maize plants was observed in response to cadmium[23]. Excess cellular concentration of cadmium either inhibits the utilization of amino acid or promotes protein hydrolysis, affecting the normal balance of cellular proteins[24] . An increase in cystiene, decrease in chlorophyll and protein content is a consequence of mercury toxicity at higher metal concentration and increased exposure in *Bacopa monnieri* plants[25] Mercury and Cadmium on amino acid content of Hydrilla plant is causing opposite effect[19] . They found cadmium treated plants contained higher amount of free amino acids.

There was an increase in DNA content of shoots and roots of the pigeon pea seedlings at 1 mgl^{-1} and 5mgl^{-1} Mercuric Chloride treatment. With further increase in concentrations of $HgCl_2$ to 10mgl^{-1} and 20mgl^{-1}, the DNA content decreased. A significant negative correlation was found in between the Cadmium Chloride treatment and DNA content of shoot and root of pigeon pea seedlings. An increase in the DNA content and RNA content in the root tips of *vicia faba*, under treatment of Cd and Zn at low concentrations was also reported by researchers.[26].

Conclusion

The present study shows that the heavy metal (Hg and Cd) stress significantly reduce the biochemical constituents of the pigeon pea seedlings. The inhibitory effect is more pronounced in $CdCl_2$ treatment compared to ($HgCl_2$).

Acknowledgement

Authors are thankful to Principal, Khallikote (A) College, Berhampur, Odisha for extending laboratory facilities and encouragements for research activities. Smt .A. Patnaik grateful to Director (Higher Education), Bhubaneswar, Odisha for awarding Teacher fellowship and also to Principal, M. M. Mahavidyalaya, Berhampur, Odisha for encouragements for research activities.

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