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EFFECT OF LEAD AND CADMIUM STRESS ON BIOCHEMICAL PARAMETERS AND ANTIOXIDANT ACTIVITY IN GERMINATED SEEDLINGS OF *CICER ARIETINUM* L.

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Abstract

The goal of the present study was to compare the biochemical parameters *viz*. (protein, crude fiber, crude fat, total carbohydrate and moisture content), enzymatic antioxidants *viz*. (superoxide dismutase, ascorbate peroxidae glutathione reductase and catalase) and non enzymatic antioxidant *viz*. (total phenol, ascorbic acid, DPPH inhibition and proline). *Cicer arietinum* L. *cv* Radhey under heavy metal (lead and cadmium) treatment. Plants were subjected to 25 ppm, 100 ppm, 150 ppm and 200 ppm heavy metal treatments for 7 days, 14 days and 21 days compared to controls. The biochemical activity (protein, crude fat, crude fiber, total carbohydrate and moisture content) showed an decrease with the increasing level of lead and cadmium and day by day increase the activity and antioxidant activity (superoxide dismutase, ascorbate peroxidase, glutathione reductase and catalase) is showed an increase with the increasing level of lead and cadmium and increase the day by day increase the activity and non enzymatic antioxidant (ascorbic acid, total phenol, DPPH and proline) is showed an increase with the increasing level of lead and cadmium the activity and day by day decrease the activity. Therefore, *Cicer arietinum* L. *cv* Radhey is considered as moderate tolerant cultivar.

Key words : *Cicer arietinum* L., seedlings, lead nitrate, cadmium nitrate, biochemical parameters, enzymatic antioxidants, non- enzymatic antioxidants.

Introduction

Chickpea (*Cicer arietinum* L.) is an important pulse crop grown and consumed all over the world. Seeds of chickpea are valuable source of protein. It is also an important source of carbohydrates, B-group vitamins, and certain minerals, particularly to the populations of developing nation and it is mostly consumed as dhal, whole seeds and several types of traditional, fermented, deep fried, sweetened, and puffed products (Chavan *et al*., 1986).

Heavy metals are significant environmental pollutants, and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons. The term "Heavy metals" refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration (Lenntech, 2004). Contamination of agricultural lands caused by heavy metals in and around industrial areas is a serious problem. Such contamination is largely due to injudicious anthropogenic activities such as indiscriminate use of pesticides containing heavy metals in agriculture, discharge of untreated industrial wastes and effluents, faulty waste disposal, high rate of burning of fossil fuels, mining etc. The presence of excessive amounts of cadmium in soil and water causes a range of plant responses, including leaf chlorosis, stunted growth, reduced photosynthesis, stimulation of stomata opening at lower concentrations, reduced plant fresh and dry mass and stomata conductance, and even death. With the development of modern industry and agriculture, Cd (cadmium) has become one of the most harmful and widespread pollutants in agricultural soils and the soil plant- environment system, mainly due to industrial emission. Application of Cd-containing sewage sludge and phosphate fertilizers and municipal waste disposal are culprits. As yet, it has been demonstrated that Cd has no biological function in plants. However, it was reported that Cd is accumulated by many cereals, potatoes, pulses, vegetables and fruits and that humans take up at least 70% of the Cd that originates from plant food. Heavy

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metal contamination has disastrous effects on plant productivity and threatens human and animal health (Adriano, 2001). Lead in the environment can cause serious problems to plants and animals. It has become a major environmental contaminant following rapid industrialization and urbanization. Lead is not amongst the essential elements for plants, but they absorb this metal if it is present in their environment, especially in rural areas where the soil is polluted by automotive exhaust and in fields contaminated with fertilizers which contain heavy metals as impurities (Adriano, 2001). Lead effects on plants have been described in several reviews (Sharma and Dubey, 2005; Sengar *et al*., 2008; Seregin and Kosevnikova, 2008).

Materials and Methods

Experimental design

For this experiment, Completely Randomized Design was used as shown in table 1. The following treatment combinations were used to provide heavy metal stress:

S. no.	Treatments	S. no.	Treatments
(T_0)	**Control (T_0)**		
	Treatments of $PbNo_3$		**Treatments of $CdNo_3$**
(T_1)	$PbNo_3$ 50 ppm	**(T_5)**	$CdNo_3$ 50 ppm
(T_2)	$PbNo_3$ 100 ppm	**(T_6)**	$CdNo_3$ 100 ppm
(T_3)	$PbNo_3$ 150 ppm	**(T_7)**	$CdNo_3$ 150 ppm
(T_4)	$PbNo_3$ 200 ppm	**(T_8)**	$CdNo_3$ 200 ppm

Sample collection

Cicer arietinum L. seeds of Radhey variety were obtained from certified seed store of local market of Alopibagh, Allahabad.

Growth conditions

Initially, *Cicer arietinum* L. seeds were taken for study and treated with Lead nitrate and Cadmium nitrate ranging from 50-200 ppm in disposable glass containing moist cotton (approx. 5ml). All treatment combinations were carried out on 7th, 14th and 21st days of seed germination.

Biochemical parameters

The following parameters will be analyzed under lead and cadmium stress in *Cicer arietinum* L. seedlings:

I. Protein content (Lowry *et al*., 1951)

II. Crude Fat (AOAC, 2000)

III. Crude Fiber (AOAC, 2000)

IV. Total Carbohydrate (Bemiller *et al*., 1998)

V. Moisture content (Windee, 1965).

Antioxidant enzymes

I. Superoxide dismutase (SOD)(Dhindsa *et al*., 1981)

II. Ascorbate peroxidase (APX) (Chen *et al*., 1989)

III. Glutathione Reductase (GR) (Catillo *et al*., 1984)

IV. Catalase (CAT) (Aebi, 1984)

Non-enzymatic antioxidants

I. Ascorbic acid (Mukherjee *et al*., 1983)

II. Total Phenol Content (Bray and Thorpe, 1954)

III. DPPH inhibition (Yen and Duh, 1994)

IV. Proline (Bates *et al*., 1973)

Results and Discussion

The protein content in the Radhey variety of *Cicer arietinum* L. seedlings showed an increasing trend day wise (*i.e.* on 7th, 14th and 21st day) whereas a declining trend was observed when the seedlings were subjected to heavy metal (lead and cadmium) stress treatments. In this cultivar protein content showed highest value at Pb 200 ppm and Cd 200 ppm and lowest value at Pb25 ppm and Cd 25 ppm. Likewise Rahman *et al.* (2008) reported that protein content was 54.91µg/g observed in *Cicer arietinum* L.

The crude fat content in the Radhey variety of *Cicer arietinum* L. seedlings showed an increasing trend day wise (*i.e.* on 7th, 14th and 21st day) but a declining trend was observed when the seedlings were subjected to heavy metal (lead and cadmium) stress treatments. In this cultivar, for crude fat the lowest value was observed in Pb 200 ppm and Cd 200 ppm and highest value was observed in Pb 25 ppm and Cd 25 ppm. Similarly, Anbreen *et al.* (1999) also reported that crude fat was 3.9 mg/ 100g seed of *Cicer arietinum* L.

The content of crude fiber in the Radhey variety of *Cicer arietinum* L. seedlings showed an increasing trend day wise (*i.e.* on 7th, 14th and 21st day) whereas a declining trend was observed when the seedlings were subjected to heavy metal (lead and cadmium) stress treatments. In this cultivar, crude fiber recorded lowest value at Pb 200 ppm and Cd 200 ppm **(T_8)** and highest value was observed at Pb 25 ppm and Cd 25 ppm. Accordingly Anbreen *et al.* (1999) reported that total crude fiber was 4.5 mg/ 100g observed in *Cicer arietinum* L.

The change in total carbohydrate content in the Radhey variety of *Cicer arietinum* L. seedlings showed an increasing trend day wise (*i.e.* on 7th, 14th and 21st day), but a declining trend was observed when the seedlings were subjected to heavy metal (lead and

Table 2 : Effect of lead and cadmium stress on biochemical parameters in germinated seedlings of *Cicer arietinum* L.

Treatment	Protein (µg/g)			Crude Fat (mg/100g)			Crude Fiber (mg/100g)			Total Carbohydrate (mg/100g)			Moisture content (%)		
	7th Day	14th Day	21st Day	7th Day	14th Day	21st Day	7th Day	14th Day	21st Day	7th Day	14th Day	21st Day	7th Day	14th Day	21st Day
Control	60	63	70	4.71	8.21	12.01	5.71	10.21	15.01	0.564	1.01	1.514	11.01	8.87	6.74
Pb 50	48	51	55	4.68	8.14	11.51	5.68	10.14	14.51	0.321	0.724	1.012	10.06	8.24	6.01
Pb 100	42	47	50	4.41	8.06	11.31	5.41	10.06	14.31	0.301	0.715	1.001	10.01	8.13	5.79
Pb 150	40	43	48	4.15	7.94	11.11	5.15	9.94	14.11	0.275	0.675	0.977	9.97	8.01	5.74
Pb 200	36	40	44	3.99	7.72	11.02	4.99	9.72	14.02	0.264	0.641	0.941	9.8	7.95	5.6
Cd 50	46	49	54	5.01	9.15	11.98	6.01	10.15	14.98	0.314	0.714	1.009	10.04	8.45	6.21
Cd 100	40	44	49	4.81	8.74	11.73	5.81	9.74	14.73	0.295	0.678	1	10	8.04	6.04
Cd 150	37	41	46	4.54	8.55	11.57	5.54	9.55	14.57	0.269	0.645	0.952	9.85	7.97	5.88
Cd 200	30	34	40	4.12	8.02	11.24	5.12	9.02	14.24	0.255	0.61	0.911	9.74	7.45	5.74
F- test	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
S. Ed. (±)	0.638	0.626	0.651	0.273	0.394	0.313	0.239	0.273	0.303	0.036	0.095	0.11	0.27	0.306	0.284
C. D. (P = 0.05)	1.317	1.292	1.343	0.564	0.814	0.646	0.493	0.563	0.625	0.074	0.195	0.227	0.558	0.631	0.586

cadmium) stress treatments. In this cultivar, total carbohydrate recorded the lowest value at Pb 200 ppm and Cd 200 ppm and highest value was observed at Pb 25 ppm and Cd 25 ppm. Likewise Chibbar *et al.* (2004) examined the similar result in *Cicer arietinum* L. was 0.465 mg/100g of total carbohydrate in sample treated with Pd with the concentration of 100 ppm after 14th day of experiment.

The moisture content in this Radhey variety of *Cicer arietinum* L. showed a declining trend in the moisture content day wise (*i.e.* on 7th, 14th and 21st day) as well as on heavy metal (lead and cadmium) stress treatments. In this cultivar the moisture content recorded lowest value at Pb 200 ppm **(T_4)** and Cd 200 ppm **(T_8)** and highest value was observed at Pb 25 ppm **(T_1)** and Cd 25 ppm **(T_5)**. Similarly Anbreen *et al.* (1999) also reported that the moisture content was 9.30% in *Cicer arietinum* L.

Hence, the biochemical parameters *viz*., (protein, crude fiber, crude fat, total carbohydrate and moisture content) in general showed a declining trend with the increasing level of lead and cadmium stress and a day wise increase in their content.

The activity of superoxide dismutase in the Radhey variety of *Cicer arietinum* L. showed an increasing trend day wise (*i.e.* on 7th, 14th and 21st day) as well as when the seedlings were subjected to heavy metal (lead and cadmium) stress treatments. In this cultivar superoxide dismutase showed the highest value at Pb 200 ppm **(T_4)** and Cd 200 ppm **(T_8)** and lowest value at Pb 25 ppm **(T_1)** and Cd 25 ppm **(T_5)**. The results of the present study were similar to Kumar *et al.* (2012), where they reported 253.50 µg/g superoxide dismutase activity in *Cicer arietinum* L. on 30 days germination period.

The activity of ascorbate peroxidase in the Radhey variety of *Cicer arietinum* L. showed an increasing trend day wise (*i.e.* on 7th, 14th and 21st day) as well as when the seedlings were subjected to heavy metal (lead and cadmium) stress treatments. In this cultivar ascorbate peroxidase showed the highest value at Pb 200 ppm **(T_4)** and Cd 200 ppm **(T_8)** and lowest value at Pb 25 ppm **(T_1)** and Cd 25 ppm **(T_5)**. Kumar *et al.* (2012) examined the similar result in *Cicer arietinum* L. was 13.8 µg/g of ascorbate peroxidase in sample treated with Pb and Cd with the control.

The activity of glutathione reductase in the Radhey variety of *Cicer arietinum* L. showed an increasing trend day wise (*i.e.* on 7th, 14th and 21st day) and also on heavy metal (lead and cadmium) stress treatments. In this cultivar glutathione reductase recorded the highest value at Pb 200 ppm **(T_4)** and Cd 200 ppm **(T_8)** and lowest value was observed at Pb 25 ppm **(T_1)** and Cd 25 ppm **(T_5)**. According to Srivastava *et al.* (2011) 5.1 µg/g total glutathione reductase was found in soybean.

The activity of catalase in this Radhey variety of *Cicer arietinum* L. there was an increasing trend day wise (*i.e.* on 7th, 14th and 21st day) in the catalase activity and also when seedlings were subjected to heavy metal (lead and cadmium) stress treatments. In this cultivar

Table 3 : Effect of lead and cadmium stress on enzymatic antioxidants in germinated seedlings of *Cicer arietinum* L.

Treatments	SOD (µg/g)			APOX (µg/g)			GR (µg/g)			CAT (µg/g)		
	7th Day	14th Day	21st Day	7th Day	14th Day	21st Day	7th Day	14th Day	21st Day	7th Day	14th Day	21st Day
Control	15	17	19	6.87	13.14	20.33	3.07	6.14	9.06	54.7	77.01	91.74
Pb 50 ppm	18	20	23	7.15	14.17	20.66	4.75	7.04	10.24	57.66	82.74	94.21
Pb 100 ppm	20	23	26	7.31	14.44	21.47	4.92	7	10.32	69.33	87.09	97.21
Pb 150 ppm	23	25	29	7.54	14.65	21.76	5.77	8.74	11.32	71.1	90.21	103.21
Pb 200 ppm	28	31	35	8.47	15.78	22.33	6.92	9.83	12.74	79	94.64	107.24
Cd 50	17	20	24	7.46	14.54	20.33	4.88	7.87	10.43	70.01	94.17	99.14
Cd 100 ppm	21	22	28	8.25	15.59	21.06	5.14	8.96	11.51	72	96.24	104.05
Cd 150 ppm	25	29	33	8.46	15.7	21.45	5.75	8.78	11.03	80.66	98.97	108.27
Cd 200 ppm	29	32	36	8.81	15.91	22.07	6.07	9.47	12.31	89.33	101.28	117.24
F- test	S	S	S	S	S	S	S	S	S	S	S	S
S. Ed. (±)	0.461	0.484	0.577	0.597	0.324	0.408	0.473	0.528	0.663	0.688	0.738	0.764
C. D. (P = 0.05)	0.952	0.999	1.192	1.233	0.668	0.843	0.975	1.089	1.369	1.42	1.524	1.576

Table 4 : Effect of lead and cadmium stress on non-enzymatic parameter in germinated seedlings of *Cicer arietinum* L.

Treatments	Ascorbic acid (µg/g)			Total phenol content(mg/g)			DPPH (mg/100g)			Proline mg/g		
	7th Day	14th Day	21st Day	7th Day	14th Day	21st Day	7th Day	14th Day	21st Day	7th Day	14th Day	21st Day
Control	2.1	4.58	6.5	13.14	6.87	3.07	5.07	3.48	2.97	1.05	1.191	1.411
Pb 50	4.02	6.25	8.54	14.17	7.15	4.75	4.85	3.12	2.75	1.426	1.875	1.981
Pb 100	5.22	7.65	8.84	14.44	7.01	4.92	4.57	3.01	2.61	1.536	1.967	2.104
Pb 150	5.97	7.97	8.98	14.85	7.54	5.77	4.24	2.88	2.42	1.65	2.102	2.252
Pb 200	3.26	6.15	8.45	15.78	8.47	6.92	4.01	2.62	2.1	1.805	2.301	2.526
Cd 50	4.14	6.28	8.35	14.54	7.46	4.88	4.52	3.08	2.6	1.837	2.314	2.521
Cd 100	5.01	7.26	8.99	15.59	8.25	5.14	4.29	2.94	2.39	1.958	2.412	2.558
Cd 150	5.75	7.87	9.21	15.7	8.46	5.75	4.09	2.73	2.14	2.011	2.524	2.712
Cd 200	4.01	6.19	8.21	15.91	8.81	6.07	3.97	2.41	2.01	2.109	2.625	2.949
F- test	S	S	S	S	S	S	S	S	S	S	S	S
S. Ed. (±)	0.507	0.53	0.553	0.378	0.271	0.325	0.233	0.19	0.124	0.289	0.379	0.398
C. D. (P = 0.05)	1.046	1.094	1.142	0.78	0.559	0.67	0.481	0.392	0.256	0.596	0.781	0.822

catalase showed highest value at Pb 200 ppm **(T_4)** and Cd 200 ppm **(T_8)** and lowest value was observed at Pb 25 ppm **(T_1)** and Cd 25 ppm **(T_5)**. Purushothaman *et al.* (2011) examined the similar result in *Cicer arietinum* L. was 35.01 µg/g of catalase.

Hence, antioxidant activity (superoxide dismutase, ascorbate peroxidase, glutathione reductase and catalase) increased day wise as well as with the increasing level of lead and cadmium stress.

The ascorbic acid content in Radhey variety of *Cicer arietinum* L. showed an increasing trend day wise (*i.e.* on 7th, 14th and 21st day) and also when seedlings were subjected to heavy metal (lead and cadmium) stress treatments. In this cultivar ascorbic acid recorded the highest value at Pb 150 ppm **(T_3)** and Cd 150 ppm **(T_7)** and lowest value was observed at Pb 25 ppm **(T_1)** and Cd 25 ppm **(T_5)**. Chavan *et al.* (1986) also reported the ascorbic acid was 2.41 µg/g seedlings of *Cicer arietinum* L.

The total phenol content in the Radhey variety of *Cicer arietinum* L. recorded a day wise (*i.e.* on 7th, 14th and 21st day) declining trend whereas an increasing trend was observed when the seedlings were subjected to heavy metal (lead and cadmium) stress treatments. In this cultivar total phenol recorded highest value at Pb 200 ppm **(T_4)** and Cd 200 ppm **(T_8)** and lowest value at Pb 25 ppm **(T_1)** and Cd 25 ppm **(T_5)**. Accordingly Sreeramulu *et al.* (2009) reported that the total phenol content was 12.75 mg/g observed in *Cicer arietinum* L.

The DPPH activity in Radhey variety of *Cicer arietinum* L. recorded a declining trend day wise (*i.e.* on 7th, 14th and 21st day) as well as on heavy metal (lead and cadmium) stress treatments. In this cultivar DPPH activity recorded the lowest value at Pb 200 ppm **(T_4)** and Cd 200 ppm **(T_8)** and highest value at Pb 25 ppm **(T_1)** and Cd 25 ppm **(T_5)**. Tom *et al.* (2013) documented similar results in *Cicer arietinum* L. which was 4.83 mg/100g of DPPH.

The proline content in Radhey variety of *Cicer arietinum* L. showed an increasing trend day wise (*i.e.* on 7th, 14th and 21st day) and also when seedlings were subjected to heavy metal (lead and cadmium) stress treatments. In this cultivar, proline recorded the highest value at Pb 200 ppm **(T_4)** and Cd 200 ppm **(T_8)** and lowest value was observed at Pb 25 ppm (T_1) and Cd 25 ppm **(T_5)**. Accordingly to Bayat *et al.* (2013) 1.012 mg/g proline content was found in *Cicer arietinum* L.

Conclusion

Hence from the above study, it can be concluded that Radhey variety of *Cicer arietinum* L. can be considered as a moderate tolerant cultivar. The antioxidant enzyme activity and non enzymatic antioxidants like ascorbic acid, total phenol and proline content increased with increasing heavy metal stress indicating the induction of antioxidant defense mechanism for self-defense, although it may not cope up fully with the production of ROS during heavy metal stress.

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