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ACTION OF LEAD AND CADMIUM STRESS ON INHIBITOR'S ACTIVITY IN GERMINATED SEEDLINGS OF CHICKPEA PLANT

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ABSTRACT

Chickpea or *Cicer arietinum* L. were drawn the attention of scientists since they are a core supply of bound minerals, carbohydrates, and B-group vitamins. Dry peas seeds and dry beans of chickpea square play an important role in measuring out valuable supply of macromolecule. The existence of heavy metals such as Cadmium and lead in water and soil impacts plants and threatens its growth. They reduce photosynthesis, a leaf chlorosis, and stunted growth of the plant. It also affects the human life since plants are one of the sources for living. The current study aims to evaluate the antioxidant enzymes and non-enzymatic antioxidants of *Cicer arietinum* L. seedlings grown under Lead and Cadmium stress. Based on the result, variety of *Cicer arietinum* L. has been shown a mild resistant cultivar indicating the induction of antioxidant defense mechanism for self-defense despite that they might not be accompanied with the production of ROS during heavy metal stress.
Keywords: Heavy metals, Environmental pollutants, Inhibitor, Agricultural lands, Enzymatic Antioxidants

Introduction

Chickpea (*Cicer arietinum* L.) is one of the most essential food sources, and it is mainly consumed in Afro-Asian countries. It is considered to be the third most important of grain legumes (Bahl and Salimath, 1996) (Jukanti *et al*., 2012). The value of chickpea seeds is important as they are a source of protein, B-group vitamins, carbohydrates, and certain minerals. They are consumed as whole seeds, dhal, and many others. Heavy metals cause environmental pollutants, and they spread toxicity in the ecological system. A heavy metal can be defined as a metallic element that is toxic and very dense even at low concentration (Lenntech, 2004). Contaminated agricultural lands by heavy metals, that is attributed to human activities such as impropriate use of pesticides contain heavy metals and an increment of burning of fossil fuels, are a controversial problem around the globe. The up- normal existence of cadmium metal in water and soil could impact the plant by causing a leaf chlorosis, reduced photosynthesis, stunted growth, etc. Heavy metals could be a threat to human and plant lives. Lead became an environmental problem and threat to animals and plants especially in urban regions. Lead is not a core element to plants; however, it is very likely to be absorbed by a plant if it exists in its environment. Rural places are a good environmental growth to Lead contamination due to the contaminated soil by heavy metals fertilizers and automotive exhaust (Chavan *et al*., 1986)

Aims of the Study

The current study aims to assess:

- The antioxidant enzymes of *Cicer arietinum* L. seedlings grown under Lead and Cadmium stress
- The non-enzymatic antioxidants of *Cicer arietinum* L. seedlings grown under Lead and Cadmium stress

Materials and Methods

Sample Collection

The sample (*Cicer arietinum* L. seeds of Radhey variety) have been collected from a trustworthy seed store in Allahabad.

Growth Circumstances

The *Cicer arietinum* L. seeds used for the study were remedied with $Cd(NO_3)_2$ and $Pb(NO_3)_2$ at range between (50-200 ppm) utilizing a wet cotton in a glass. The treatments were conducted on days (7th, 14th, and 21st) of seed germination (A.O.A.C. 2000).

Treatment Combinations

Aqueous cadmium and led nitrate were used for the study as their structural formulas are shown in figure (1).

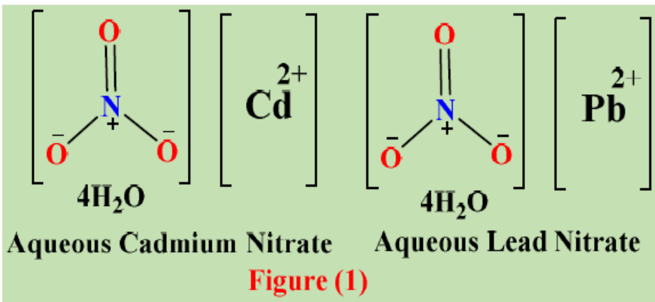

Fig. 1 : The compounds used for the analysis

While the treatment combinations in table (1) have been utilized to run heavy metal stresses:

Table 1 : The treatment combinations used to provide heavy metal stress

S. No.	Treatments of $PbNO_3$	S. No	Treatments of $CdNO_3$
(T1)	50 ppm	(T5)	50 ppm
(T2)	100 ppm	(T6)	100 ppm
(T3)	150 ppm	(T7)	150 ppm
(T4)	200 ppm	(T8)	200 ppm
(T0)	Control		

Assay of Enzymatic antioxidants

(i) Superoxide Dismutase (SOD)

The dissociation of superoxide radical (O_2) to H_2O_2 is catalyzed by SOD. The principle of this assay to form formazone by the reaction of O_2 radical and nitro-blue tetrazolium that absorbs at 560 nm. SOD enzyme reduces the absorption due to the above reaction. The blank yields highest absorbance, that is reduced as an increment of enzyme activity takes place (Dhindsa *et al.*, 1981)

(ii) Ascorbate Peroxidase (APX)

(APX) transforms H_2O_2 to water by reduction in the presence of ascorbic acid. After producing mono-dehydro ascorbic acid by oxidation, dehydroascorbate is obtained by metabolization. A decrease in the absorption of ascorbic acid at 290 nm is because of the oxidation that occurred in the reaction above (Nakano and Asada, 1981).

(iii) Glutathione Reductase

The reduction of Glutathione catalyzes the reduction of oxidized glutathione (GSSG) to reduce glutathione (GSHG) by utilizing a reducing agent NADPH.

(iv) Catalase

Catalase induces the reaction of H_2O_2 to H_2O and O_2. In a comparison to (APX), it is less effective system of H_2O_2 scavenging due to a high value (km) for H_2O_2 than (APX). The assay of Catalase relied on the hydrogen peroxide absorbance at 240 nm in UV- range (Castillo *et al.*, 1984)

Assay of Non-enzymatic antioxidants

(i) Ascorbic Acid

Ascorbic acid is in charge of non-enzymatic scavenging of O_2 radical and H_2O_2 and regeneration of α-tocopherol in chloroplast. Its assay relies on a pink complex as a result of a reduction process of dinitrophenyl hydrazine using ascorbic acid to form phenyl hydrazine (Mukherjee and Choudhari, 1983).

(ii) Assessment of Total Phenols

Blue Molybdenum is resulted when Phenols interact with phosphomolybdate (an oxidizing agent) in alkaline media using Folin-Ciocalteu reagent. The Molybdenum is scored at 650 nm calorimetrically (Brayand Thorpe, 1954)

(iii) Estimation of DPPH inhibition:

The free radical scavenging activity of DPPH was conducted by Yen and a coworker (Yen. and Duh, 1994).

(iv) Evaluation of proline

Through the extraction process using aqueous sulphosalicylic acid, proteins were obtained as a complex. Any unwanted materials were disposed of by absorption to protein sulphosalicylic acid complex. The resulted proline is used to interact with ninhydrin under acidic circumstances (pH = 1.0) and produce a red color substance (chlorophore). The reagents used were (acid ninhydrin, aqueous sulphosalicylic acid, glacial acetic acid, Toluene, and concentrated proline solution)(Bates et. al. ,1973).

Extraction of Protein from sample

Using a (10 ml) buffer solution, 500 mg of the sample were dissolved after they went through grinding process by a pestle and mortar. For protein estimation, the samples were centrifuges and sedimented using a centrifuge. Standard graph was plotted, and the amount of the protein was quantified. μ moles of proline were calculated using following formula:

$$\mu\text{moles of } \frac{\text{Proline}}{\text{g}} \text{ tissue} = \frac{\mu\text{g proline /ml} \times \text{ml toluene}}{115.5} X \frac{5}{\textit{Sample (g)}}$$

Where:

115.5 is the molecular weight of proline.

Results and Discussion

There was an increasing trend (day wise, i.e. on 7th, ordinal and twenty first day) in SOD activity whereas an increasing trend was ascertained once the seedlings were subjected to serious metal (lead and cadmium) stress treatments. Throughout the study, the results acquired that have been shown the stress of heavy metals on enzymes activities as they are explained bellow:

Table (2) shows the activity of superoxide dismutase (SOD) in Radhey variety of *Cicer arietinum* L. seeds. Subjecting the seedlings to (Ca and Pb) stress treatment was observed elevation tendency, whereas an increasing tendency (on 7th, 14th and 21st day) in SOD activity. In this cultivar of (SOD), the (Pb) value was high 200 ppm (T4) and Cd 200 ppm (T8), while lowest value at Pb 25 ppm (T1) and Cd 25 ppm (T5).

Table 2 : Effect of lead and cadmium stress on superoxide dismutase (SOD) in germinated seedlings of *Cicer arietinum* L.

Treatment code	7^{th} Day	14^{th} Day	21^{st} Day
T0	15	17	19
T1	18	20	23
T2	20	23	26
T3	23	25	29
T_4	28	31	35
T_5	17	20	24
T_6	21	22	28
T_7	25	29	33
T_8	29	32	36
F-test			
S. Ed. (±)	0.461	0.484	0.577
C.D. (P =0.05)	0.952	0.999	1.192

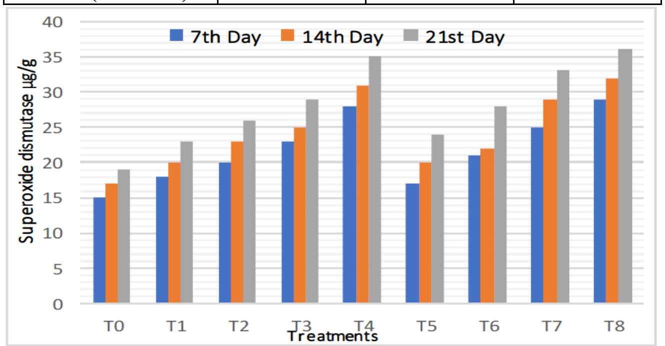

Fig. 2 : Effect of lead and cadmium stress on superoxide dismutase (SOD) in germinated seedlings of *Cicer arietinum* L.

The activity of ascorbate peroxidase (APX) in the Radhey variety of *Cicer arietinum* L. seeds was shown in table (3). Subjecting the seedlings to (Ca and Pb) stress treatment was observed an elevation tendency, whereas an increasing tendency (on 7th, 14th and 21st days) in APX activity. In this cultivar of (ascorbate peroxidase), the (Pb) value was scored high 200 ppm (T4) and Cd 200 ppm (T8), while lowest value at Pb 25 ppm (T1) and Cd 25 ppm (T5).

Table 3 : Effect of lead and cadmium stress on ascorbate peroxidase (APX) in germinated seedlings of *Cicer arietinum* L.

Treatment code	7th Day	14th Day	21st Day
T0	6.87	13.14	20.33
T1	7.15	14.17	20.66
T2	7.31	14.44	21.47
T3	7.54	14.65	21.76
T_4	8.47	15.78	22.33
T_5	7.46	14.54	20.33
T_6	8.25	15.59	21.06
T_7	8.46	15.70	21.45
T_8	6.87	13.14	20.33
F-test			
S. Ed. (±)	0.597	0.324	0.408
C.D. (P =0.05)	1.233	0.668	0.843

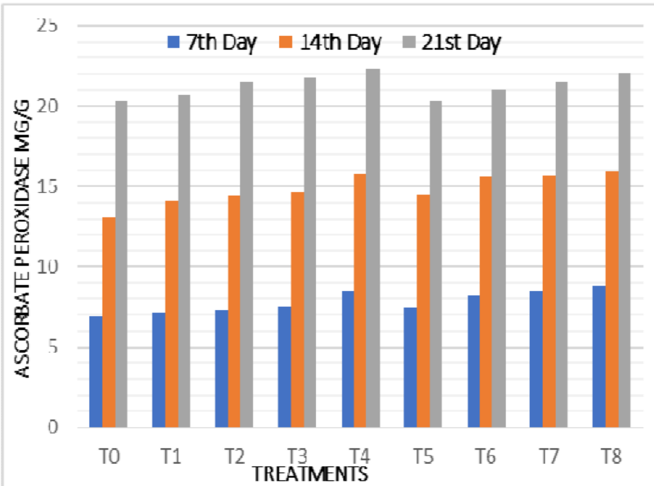

Fig. 3 : Effect of lead and cadmium stress on ascorbate peroxidase (APX) in germinated seedlings of *Cicer arietinum* L.

The glutathione reductase activity in the Radhey variety of *Cicer arietinum* L. seeds was shown in table (4). Subjecting the seedlings to (Ca and Pb) stress treatment was scored as elevation tendency, whereas it was shown an increasing tendency (on 7th, 14th and 21st day) in glutathione reductase activity. In this cultivar of glutathione reductase, the (Pb) value was high 200 ppm (T4) and Cd 200 ppm (T8), while lowest value at Pb 25 ppm (T1) and Cd 25 ppm (T5). Based on the investigation that was conducted by Srivastava and coworkers, (Srivastava. et. al 2011), it was found that soybean contains about 5.1 µg/g total glutathione reductase.

Table 4 : Effect of lead and cadmium stress on glutathione reductase (GR) in germinated seedlings of *Cicer arietinum* L.

Treatment Code	7th Day	14th Day	21st Day
T0	3.07	6.14	9.06
T_1	4.75	7.04	10.24
T_2	4.92	7.00	10.32
T_3	5.77	8.74	11.32
T4	6.92	9.83	12.74
T_5	4.88	7.87	10.43
T_6	5.14	8.96	11.51
T_7	5.75	8.78	11.03
T_8	6.07	9.47	12.31
F- test			
S. Ed. (±)	0.473	0.528	0.663
C. D. (P =0.05)	0.975	1.089	1.369

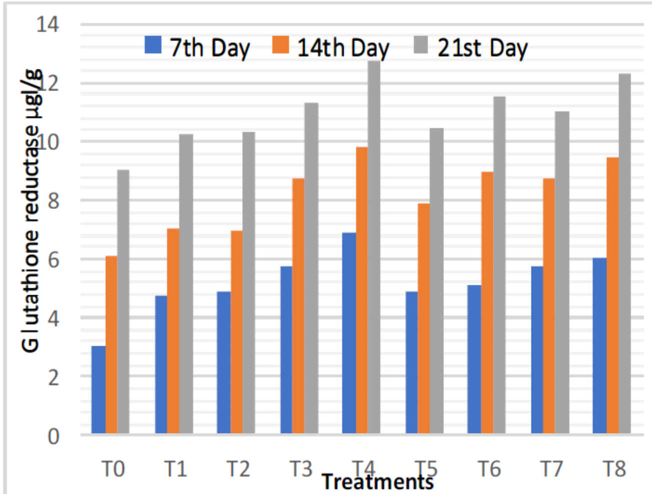

Fig. 4 : Effect of lead and cadmium stress on glutathione reductase (GR) in germinated seedlings of *Cicer arietinum* L

The activity of catalase in the Radhey variety of *Cicer arietinum* L. seeds was shown in table (5). Subjecting the seedlings to (Ca and Pb) stress treatment was observed elevation tendency, whereas an increasing tendency (on 7th, 14th and 21st day) in APX activity. In this cultivar of catalase, the (Pb) value was scored high 200 ppm (T4) and Cd 200 ppm (T8), while lowest value was obtained for Pb at 25 ppm (T1) and Cd 25 at ppm (T5). Based on a published work by Purushothaman and others, it was proven that 35.01 µg/g of catalase was found in *Cicer arietinum* L. (Purushothaman *et al.*, 2011)

Table 5 : Effect of lead and cadmium stress on catalase (CAT) in germinated seedlings of *Cicer arietinum* L.

Treatment Code	7th Day	14th Day	21st Day
T0	54.70	77.01	91.74
T_1	57.66	82.74	94.21
T_2	69.33	87.09	97.21
T_3	71.10	90.21	103.21
T4	79.00	94.64	107.24
T_5	70.01	94.17	99.14
T_6	72.00	96.24	104.05
T_7	80.66	98.97	108.27
T_8	54.70	77.01	91.74
F- test			
S. Ed. (±)	0.688	0.738	0.764
C. D. (P =0.05)	1.420	1.524	1.576

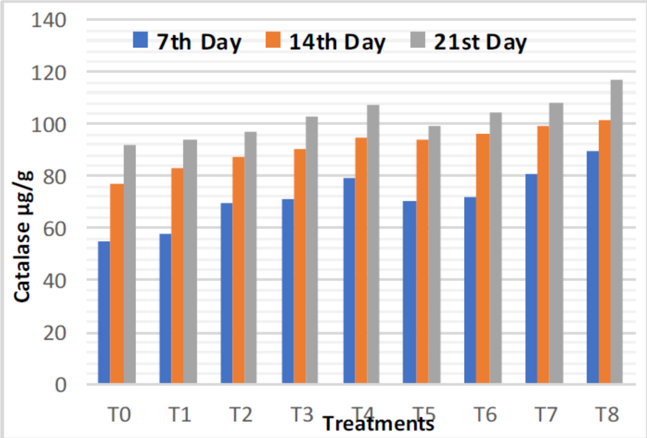

Fig. 5 : Effect of lead and cadmium stress on catalase (CAT) in germinated seedlings of *Cicer arietinum* L.

The activity of ascorbic acid in the Radhey variety of *Cicer arietinum* L. seeds was shown in table (6). Subjecting the seedlings to (Ca and Pb) stress treatment was observed elevation tendency, whereas an increasing tendency (on 7th, 14th and 21st day) in ascorbic acid activity. In this cultivar of catalase, the (Pb) value was high 150 ppm (T3) and Cd 150 ppm (T7) , while lowest value at Pb 25 ppm (T1) and Cd 25 ppm (T5). Based on a result obtained by Chavan and coworkers, it was reported that 2.41 µg/g of ascorbic acid was found *Cicer arietinum* L. (Chavan *et al*., 1986)

Table 6 : Effect of lead and cadmium stress on ascorbic acid in germinated seedlings of *Cicer arietinum* L.

Treatment Code	7th Day	14th Day	21st Day
T0	2.10	4.58	6.50
T_1	4.02	6.25	8.54
T_2	5.22	7.65	8.84
T_3	5.97	7.97	8.98
T4	3.26	6.15	8.45
T_5	4.14	6.28	8.35
T_6	5.01	7.26	8.99
T_7	5.75	7.87	9.21
T_8	2.10	4.58	6.50
F- test			
S. Ed. (±)	0.507	0.530	0.553
C. D. (P =0.05)	1.046	1.094	1.142

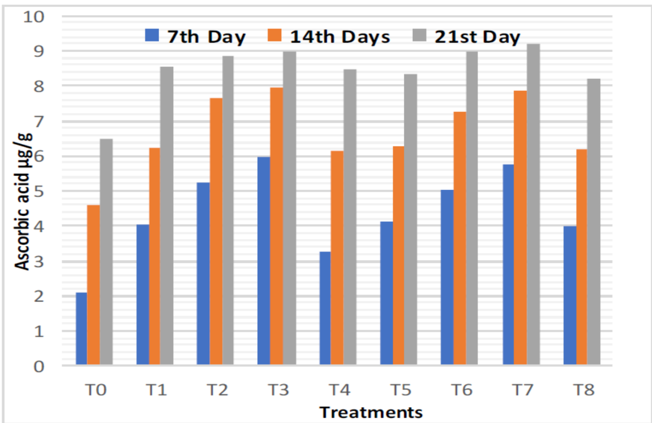

Fig. 6 : Effect of lead and cadmium stress on ascorbic acid in germinated seedlings of *Cicer arietinum* L.

The activity of phenol content in the Radhey variety of *Cicer arietinum* L. seeds was shown in table (7). Subjecting the seedlings to (Ca and Pb) stress treatment was observed elevation tendency, whereas a declining tendency was observed (on 7th, 14th and 21st day) in the total phenol content. In this cultivar of total phenol, the (Pb) value was high 200 ppm (T4) and Cd 200 ppm (T8) , while lowest value at Pb 25 ppm (T1) and Cd 25 ppm (T5).

Table 7 : Effect of lead and cadmium stress on total phenol content in germinated seedlings of *Cicer arietinum* L.

Treatment Code	7th Day	14th Day	21st Day
T0	13.14	6.87	3.07
T_1	14.17	7.15	4.75
T_2	14.44	7.01	4.92
T_3	14.85	7.54	5.77
T4	15.78	8.47	6.92
T_5	14.54	7.46	4.88
T_6	15.59	8.25	5.14
T_7	15.70	8.46	5.75
T_8	13.14	6.87	3.07
F- test			
S. Ed. (±)	0.378	0.271	0.325
C. D. (P =0.05)	0.780	0.559	0.670

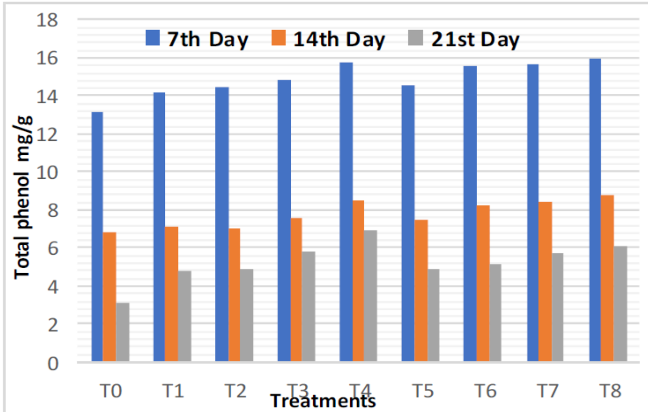

Fig. 7 : Effect of lead and cadmium stress on total phenol content in germinated seedlings of *Cicer arietinum* L.

The activity of DPPH in the Radhey variety of *Cicer arietinum* L. seeds was shown in table (8). Subjecting the seedlings to (Ca and Pb) stress treatment was observed elevation tendency, whereas a declining tendency was observed (on 7th, 14th and 21st day) in the DPPH activity. In this cultivar of DPPH activity, the (Pb) value was high 200 ppm (T4) and Cd 200 ppm (T8) , while lowest value at Pb 25 ppm (T1) and Cd 25 ppm (T5).

Table 8 : Effect of lead and cadmium stress on DPPH inhibition in germinated seedlings of *Cicer arietinum* L.

Treatment Code	7th Day	14th Day	21st Day
T0	5.07	3.48	2.97
T_1	4.85	3.12	2.75
T_2	4.57	3.01	2.61
T_3	4.24	2.88	2.42
T4	4.01	2.62	2.10
T_5	4.52	3.08	2.60
T_6	4.29	2.94	2.39
T_7	4.09	2.73	2.14
T_8	3.97	2.41	2.01
F- test			
S. Ed. (±)	0.233	0.190	0.124
C. D. (P =0.05)	0.481	0.392	0.256

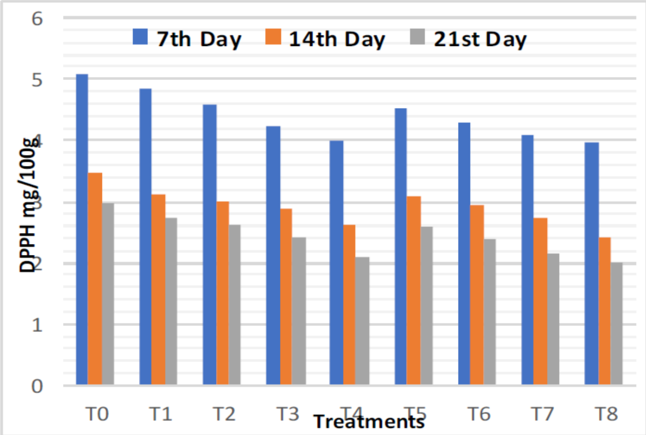

Fig. 8 : Effect of lead and cadmium stress on DPPH inhibition in germinated seedlings of *Cicer arietinum* L.

The proline content in the Radhey variety of *Cicer arietinum* L. seeds was shown in table (9). There was an elevation tendency in proline content that was observed (on 7th, 14th and 21st day), as well as, when subjecting the seedlings to (Ca and Pb) stress treatment. In this cultivar of proline, the (Pb) value was high 200 ppm (T4) and Cd 200 ppm (T8) , while lowest value at Pb 25 ppm (T1) and Cd 25 ppm (T5).

Table 9 : Effect of lead and cadmium stress on proline in germinated seedlings of *Cicer arietinum* L.

Treatment code	7^{th} Day	14^{th} Day	21^{st} Day
T0	1.050	1.191	1.411
T1	1.426	1.875	1.981
T2	1.536	1.967	2.104
T3	1.650	2.102	2.252
T_4	1.805	2.301	2.526
T_5	1.837	2.314	2.521
T_6	1.958	2.412	2.558
T_7	2.011	2.524	2.712
T_8	2.109	2.625	2.949
F-test			
S. Ed. (±)	0.289	0.379	0.398
C. D. (P = 0.05)	0.596	0.781	0.822

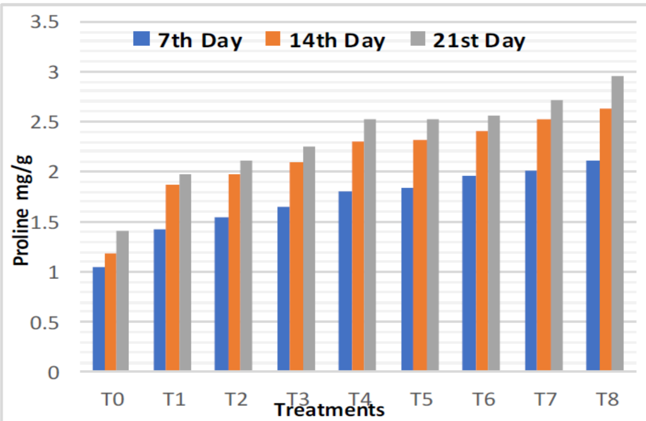

Fig. 9 : Effect of lead and cadmium stress on proline in germinated seedlings of *Cicer arietinum* L.

The significant metals are a unit important environmental pollutant, and their toxicity could be a downside of skyrocketing significance for ecological, biological process, biological process and environmental reasons. Significant metals area unit important environmental pollutants, and their toxicity could be a downside of skyrocketing significance for ecological, biological process, biological process and environmental reasons(Nagajyoti. et. al. 2010). The catalyst activeness and deterioration of seed stockpile materials in BARI-2, BARI-1, and a pair of BARI-3 pattern of *Cicer arietinum* L. seeds over a duration of various courses of upgrowth. The number of overall macromolecule and dissolved macromolecule have been exhibited high values in BARI-1 and BARI-3, severally. The starch content in chickpea seed cut step by step throughout germination. The seed stockpile material was proven to be reduced step-by-step with the rise in the emergence of up growth period. The results show that deterioration of stock seed nutrients quicken the growth of phanerogams throughout germinate ion (Sharma *et al.*, 2013; Rahman *et al.*, 2008).

Conclusion

Based on the current study and the obtained results, it can be concluded that Radhey variety of *Cicer arietinum* L. is a moderate tolerant cultivar indicating the induction of antioxidant defense mechanism for self-defense. Although, they may not come up fully with the production of ROS during heavy metal stress.

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Conflict of Interest:

I have no conflict of interest regarding this study

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