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Cadmium toxicity on Soybean (*Glycine max* L.)

Samuel J Chikile, Reeta Sharma*and N.R. Mogallapu

Department of Botany, S.V.R.K. Govt. Degree College, Nidadavole, W.G. District., A.P., India-534320.
*Department of Botany, Regional Institute of Education (NCERT), Bhopal. India-462013

ABSTRACT

This paper emphasizes effect of Cadmium nitrate on Soybean with regards to morphological and biochemical aspects. Lower concentrations of the metal nitrate were more enhancing in seed germination and early flowering. Higher concentration were more effective on seed germination, seedling growth, root elongation, root nodules, flowers, Protein, Amino acid and carbohydrate contents.

Key words: Soybean, Cadmium nitrate, Morphological, chlorophyll content, Protein Amino acid, Carbohydrate.

The potential for environmental contamination by cadmium (Cd) has increased significantly in recent years. Cadmium, a non-essential metallic element, highly toxic and negatively affects on the plant growth and development, as well as the life cycle of living organisms. The continuous inputs of this element in biosphere, as a result of various industrial activities, may pose a risk to the soil and is readily uptake by plants[9]. Cadmium occurs as a major environmental contaminant in agricultural soil, it is mainly derived from fertilizer, fungicides and some phosphatic fertilizers Anderson[1] Cadmium has been reported to be phytotoxic[16] and when taken up by animals and human being causes toxicity[6]. It is also inhibits nitrogen metabolism in higher plants[4]. Cadmium has been shown to cause many morphological, biochemical and structural changes in plants, such as growth inhibition, water imbalance and inhibition of seed germination Mishra *et al.*,[14]

Turnar[12] noted in vegetable species that Cd causes root damage, which resulted in reduced yield of crops. Haung *et al.*,[10] have observed the reduction of dry weight of roots and nodules in soybean plants. Smeyers, *et al*.[21] reported cadmium induced symptoms like root growth retardation, suberization and damage of external and internal structures of root and decreased root hydraulic water conductance in soybean. Bazzaz *et al*.[3], studied the effect of lead on photosynthesis and transpiration in corn and soybean and they found a decrease in the net photosynthesis and transpiration in these plants with increasing lead doses. Huang *et al.*,[10] noticed the depressed photosynthesis and a decrease in some enzyme activities in response to Cd treatment in soybean plants. Griffith[8] reported that cadmium inhibited the production of chlorophyll and affected on synthesis of amino acid and the protochlorophyllide reductase ternary complex with its substrate. Lee *et al.*,[12] observed decreased activity of hydrolytic enzymes in soybean plants treated with Cd. Cadmium is known to be toxic for carbohydrate metabolism and also known to inhibit carbonic anhydrase.

Cadmium causes stomatal closer in higher plants[17] and an overall inhibition of photosynthesis[11,19]. Growth reduction associated with cadmium treatment was probably caused by inhibition of protein synthesis[6]. Phytotoxicity of the metal in other crop plants has been reported in the form of loss in protein levels by Dubey and Dwivedi,[5] Moreover, the grains developed on the plants grown under Cd stress had lower protein content Salgare[18]. In this research article we focussed on the toxicity of cadmium on soybean.

The present research was carried out on soybean by using three concentrations of cadmium nitrate *i.e* 0.01M; 0.001M.0.0001M. The following parameters was studied in two crop years. Seed germination, seedling growth, morphological parameters, nodulation, Chlorophyll content and the associated biochemical changes of protein, amino acids and carbohydrate contents were carried out. The seeds were sterilized with 20% ethanol for 30seconds followed by treatment with 0.1% $HgCl_2$ for two minutes. Then washed with double distilled water then transferred for seed germination and growth. Readings were taken after eight days for seed germination, seedling growth, root length, shoot length and number of lateral roots.

Chemical treatment and application:

Stock solution (500ml of 0.1M) was prepared for each salt and diluted as per requirement before use. Plants were transferred to shade and 50ml of test solution was applied to each pot and water was applied to control plant. Plant height was recorded from the base of the plant at soil level up to the topmost expanded leaf of the main stem. The total number of leaves present on the main stem from the base to apex was recorded. The total number of flowers recorded in both treated and control plants after 49 days. The numbers of pods were recorded after 84 days. Ten plants were uprooted after 84 days and both fresh and dry weights of the nodules were recorded. 100 mg of leaf material was taken for chlorophyll content, crushed with 20ml of 80% acetone and pinch of magnesium carbonate, centrifuged and made up to 100ml by 80% acetone then O.D was recorded for estimation.

Biochemical Analysis:

The samples for Biochemical analysis were taken every seven days till the end of the experiment. Protein content was estimated by the method of Lowry *et al.*,[13] using Bovine Serum Albumin for standard curve against appropriate blank. Amino acid content was estimated by ninhydrin method that was developed by Moore and Stein[15] using the standard curve prepared with Glycine by taking appropriate blank. Carbohydrate content was estimated by Anthron method and Glucose stock solution was applied for standard curve. The data obtained for various parameters were subjected to statistical analysis, the mean values and Standard deviation was calculated.

Seed germination: The results of seed germination are presented in table-1. It is evident from the table that seed germination initiated on day two and was completed in 8 days. $Cd(NO_3)_2$ could not cause inhibition in seed germination in the initial days but as the time progressed slight inhibition was observed especially after 6 days *i.e* with higher concentration (0.01M).

Seedling growth: Seedling growth is presented in table-3. Higher concentration of the metal nitrate affected both root and lateral roots.

Stem elongation and number of pods: The results are presented in table-2. The table shows the lower concentration of metal nitrate, in stem elongation is slightly more than that of control plants. Number of pods are recorded in table 2. Pods appeared after 56 days and counted after 77 days. All the treated plants were affected on number of pods than control plants. Fresh and dry weights were recorded after 77 days. In cadmium treated plants the decrease was substantial and amounted to just half of the nodule weight of the control plants.

Chlorophyll content: Beside initial estimation, the chlorophyll contents were estimated thrice. First after seven days of start of experiment, second when the flowering was initiated and finally after 77 days when the experiment was terminated (table-6). The decreased chlorophyll content was noted in the treated plants at the final stage.

Protein content: The results of protein content were recorded in fig-1. All the treated plants were found to have decreased chlorophyll content than the control plants. Higher concentration was more effective than the lower concentrations.

Tab-1. Cadmium toxicity on seed germination of soybean (*Glycine max* L.)

Days aftertr treatment	concentration			Control
	0.01M	0.001M	0.0001M	
2	11.334 ± 1.696	16.000 ± 1.988	21.334 ± 3.457	10.667 ± 1.457
3	46.667 ± 2.134	57.334 ± 2.489	65.334 ± 4.689	49.334 ± 2.542
4	66.667 ± 6.495	78.000 ± 3.053	84.000 ± 3.053	66.000 ± 3.199
5	75.334 ± 9.614	82.667 ± 6.105	86.334 ± 5.696	76.000 ± 6.371
6	78.000 ± 9.865	84.000 ± 3.601	93.234 ± 5.896	84.000 ± 8.160
7	82.667 ± .569	90.000 ± 7.840	95.334 ± 2.613	89.000 ± 9.053
8	85.334 ± 3.457	92.667 ± 6.914	96.667 ± 2.163	94.667 ± 3.457

Values expressed in Mean ± S.D

Table-2. Cadmium toxicity on stem elongation and number of leaves of soybean (*Glycine max* L.)

Days after start of experiment	Stem elongation				Number of leaves			
	Concentration				Concentration			
	0.01M	0.001M	0.0001M	control	0.01M	0.001M	0.0001M	control
initial	6.060 ± 0.313	6.060 ± 0.313	6.060 ± 0.313	6.060 ± 0.313	2. 700 ± 0.483	2.700 ± 0.483	2.700 ± 0.483	2.700 ± 0.483
7	12.400 ± 1.145	14.800 ± 1.141	20.200 ± 1.864	12.280 ± 1.141	4.600 ± 0.884	4.900 ± 844	5.600 ± 0.546	4.800 ± 0.844
14	16.800 ± 2.828	21.200 ± 1.095	24.200 ± 0.978	19.300 ± 1.225	6.400 ± 1.140	7.200 ± 1.095	8.400 ± 1.674	6.400 ± 1.140
21	22.800 ± 1.414	24.200 ± .976	29.600 ± 2.688	28.800 ± 1.483	7.200 ± 1.095	8.300 ± 0.499	8.900 ± 1.034	8.000 ± 0.837
28	28.900 ± 2.608	36.000 ± 1.789	32.200 ± 1.817	32.000 ± 1.789	8.400 ± 0.449	9.100 ± 0.837	9.500 ± 0.150	12.000 ± 1.414
35	32.400 ± 1.784	34.400 ± 1.830	34.800 ± 1.374	40.800 ± 1.949	9.200 ± 0.837	10.200 ± 1.095	10.400 ± 0.674	12.800 ± 1.095
42	34.600 ± 1.113	35.200 ± 1.892	36.800 ± 1.742	44.400 ± 6.428	10.400 ± 0.674	10.800 ± 0.963	11.200 ± 0.837	14.800 ± 1.934
49	36.800 ± 6.313	35.200 ± 1.892	36.800 ± 1.742	48.300 ± 1.319	10.800 ± 1.219	11.400 ± 0.894	12.300 ± 1.342	18.200 ± 2.828
56	38.200 ± 1.673	37.200 ± 2.408	38.200 ± 1.315	50.400 ± 2.284	11.600 ± 1.304	12.600 ± 0.716	13.800 ± 1.342	19.300 ± 1.225
63	40.000 ± 0.828	38.800 ± 1.643	41.400 ± 2.682	51.800 ± 1.438	12.200 ± 0.447	13.300 ± 0.558	14.000 ± 1.224	20.600 ± 1.414
70	41.400 ± 1.581	42.200 ± 1.678	44.400 ± 1.156	52.600 ± 1.264	12.800 ± 1.934	13.900 ± 1.291	15.200 ± 1.200	21.800 ± 1.095
77	42.800 ± 1.688	44.200 ± 1.156	46.800 ± 0.889	54.800 ± 2.871	13.600 ± 0.713	14.800 ± 1.140	16.400 ± 1.937	11.400 ± 1.088

Values expressed in Mean ± S.D

Table-3. Cadmium toxicity on seedling growth of soybean (*Glycine max* L.)

Treatment/Metal Concentration	Shoot length	Root length	No of lateral roots
0.01M	2.445 ± 0.490	1.736 ± 0.312	2.538 ± 0.183
0.001M	3.152 ± 0.452	2.153 ± 0.283	3.607 ± 0.806
0.0001M	3.722 ± 0.351	2.764 ± 0.413	4.461 ± 0.450
Control	7.837 ± 1.103	5.987 ± 1.009	16.630 ± 1.166

Values expressed in Mean ± S.D

Table-4. Cadmium toxicity on no of flowers of soybean (*Glycine max* L.)

Days aftertr start of experiment	concentration			Control
	0.01M	0.001M	0.0001M	
21	2.400 ± 0.082	5.200 ± 0.837	5.700 ± 1.683	5.200 ± 0.837
28	4.600 ± 1.386	9.60 ± 1.678	7.20 ± 0.836	7.40 ± 1.383
35	7.600 ± 0.722	12.20 ± 1.889	11.80 ± 1.828	10.60 ± 1.517
42	8.800 ± 1.518	7.400 ± 0.894	14.600 ± 1.306	18.400 ± 2.456
49	5.200 ± 0.448	5.600 ± 0.702	8.700 ± 1.345	9.200 ± 1.245

Values expressed in Mean ± S.D

Table-5. Cadmium toxicity on fresh and dry weights of nodules (mg)
of soybean (*Glycine max* L.)

$Cd(NO_3)_2$	concentration			control
	0.01M	0.001M	0.0001M	
Fresh weight of nodules	163.91	210.22	272.10	370. 36
Dry weight of nodules	67.40	81.79	94.34	148. 34

Table-6. Cadmium toxicity on chlorophyll content (mg/g) and number of pods of soybean (*Glycine max* L.)

concen-tration	Time of estimation				Number of pods
	initial	After first weak	At the time of Flowering	At the time of harvesting	
0.01M	3.224 ± 0.302	1.321 ± 0.472	2.839 ± 0.48	6.224 ± 0.653	6.800 ± 0.702
0.001M	3.224 ± 0.302	2.432 ± 0.307	3.138 ± 0.683	8.278 ± 0.829	10.200 ± 0.268
0.0001M	3.224 ± 0.302	3.024 ± 0.599	4.214 ± 0.516	10.871 ± 0.574	12.800 ± 1.652
control	3.224 ± 0.302	6.314 ± 0.25	11.221 ± 0.686	27.224 ± 1.271	14.200 ± 0.512

Values expressed in Mean ± S.D

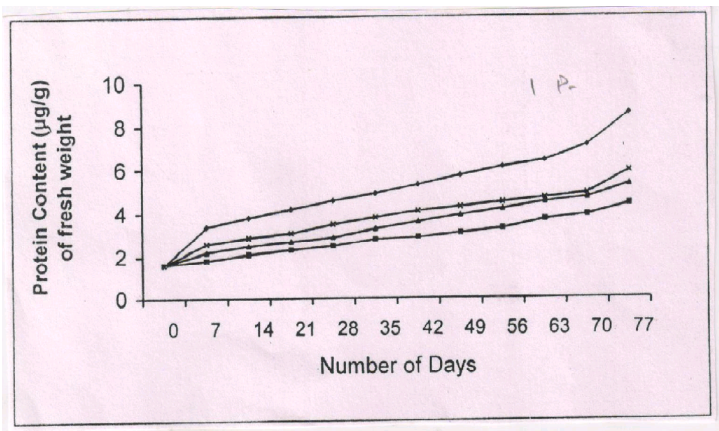

Fig-1: Cadmium toxicity on protein content (mg/gm) of Soybean (*Glycine max* L.)

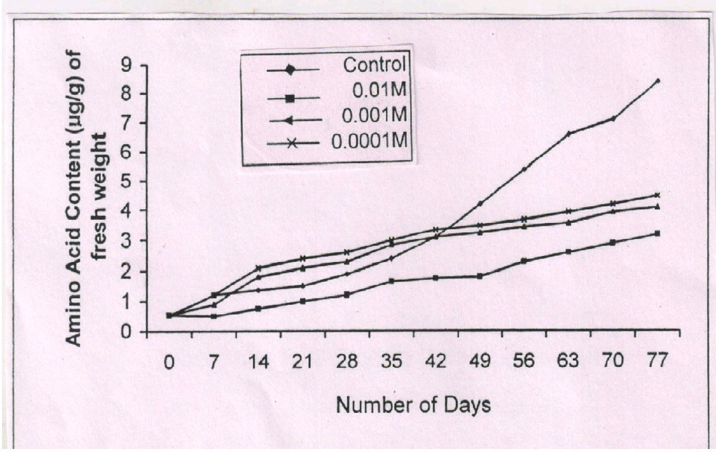

Fig-2: Cadmium toxicity on amino acid content (mg/gm) of Soybean (*Glycine max* L.)

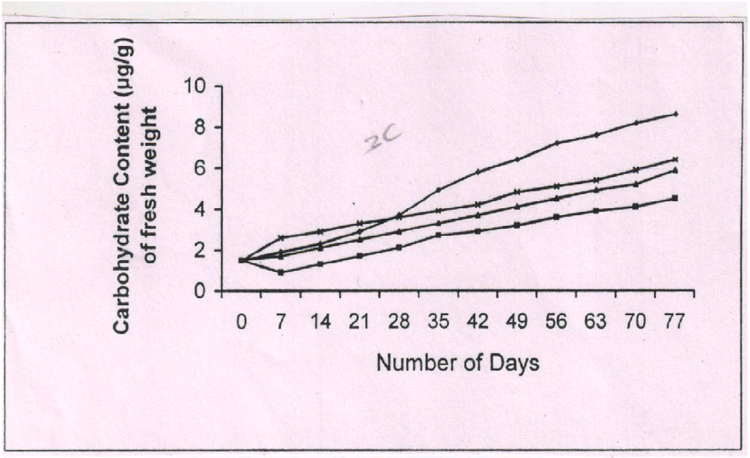

Fig-3: Cadmium toxicity on carbohydrate content (mg/gm) of Soybean (*Glycine max* L.)

Amino acid content: The results are presented in fig-2 and the higher concentrations of the metal nitrate (0.01M) treated plants, the content remained low. It is interestingly noted that the lower concentration (0.0001M) treated plants remained higher than the control plants.

Carbohydrate content: The carbohydrate content is recorded in fig-3 and it is interesting to note that under the lower concentration of the metal, the content was high than the control plants till 21 days. After that the content decreased till the end of the experiment.

The present investigation was undertaken with a view to study the effect of Cadmium nitrate on seed germination, seedling growth, leaf number, number of flowers, number of pods and nodulation of Soybean. The associated biochemical changes such as chlorophyll/ protein /amino acid and Carbohydrate contents as affected by cadmium. The present investigation has been discussed in the light of recent literature available on the subject.

Cadmium nitrate inhibited seed germination of Soybean. This inhibition is observed at higher concentrations. Several reports are available in different plants[10,21,22]. Inhibition of Cd^{2+} has been reported by Dubey and Dwivedi[5]; Mishra and Choudary[14]. All the three concentrations Cadmium nitrate decreased the root noidules (both fresh and dry weights). This decreased nodulation is reported by Huang *et al.*,[10]. The inhibition of Chlorophyll biosynthesis in higher plants has been reported by Baszinksy *et al.*, (1981). This leads to decrease in the photosynthesis and also yield. Similar effect on the metal has been reported by Sing *et al.* (1994).

Protein content decreased in all the treated plants. This decrease of protein content with higher concentrations is in conformity with the results of Singh[20]; Aesche and Clijster[2]. All the three concentrations of metal nitrate decreased the amino acid content. The heavy metals are known to bind with free amino acids to form complex that alter the initial process of translation interfere with the chain elongation process of translocation which drastically affect the cell[20].

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