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Impact of Cobalt on the Growth, Pigmental, Some Biochemical and Enzymatic Characteristics of *Eleusine coracana* (L.) Gaertn

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Abstract: Various pollutants cause different types of pollution in the environment. Among the pollutants, the heavy metals are considered as emerging pollutants in daily life of food crop plants. These heavy metals cause soil and water pollution in our biosphere. Cobalt is one of the heavy metals to cause soil and water pollution in excess. In this study, the seedlings of (finger millet) *Eleusine coracana* (L) Gaertn were treated with various concentrations of cobalt chloride and its impact on the morphometric, pigmental, biochemical and enzymatic characters was studied. After ten days of treatment with different concentrations of cobalt chloride (2, 4, 6, 8, & 10 mM), the growth parameters such as leaf area, fresh weight, dry weight, shoot length, root length were decreased compare to the control. Pigmental characters such as chlorophyll a, chlorophyll b, total chlorophylls and carotenoids content were decreased with increasing the concentrations of cobalt chloride, while the content of anthocyanin was increased of the biochemical parameters the soluble sugar and protein content were found decreased with the increase in the concentrations of cobalt chloride and the contents of free amino acid, proline and leaf nitrate were increased with increasing the concentrations of cobalt chloride. The activities of enzymes such as catalase and peroxidase were increased with increasing the concentration of cobalt chloride and the NRA enzyme activity was decreased. The Atomic Absorption of Spectroscopy (AAS) technique was employed to confirm the presence of cobalt chloride in the treated plants and control. Comparison of the values of treated plants with control reveals that cobalt chloride has seriously affected the finger millet plants.

Key words: Cobalt · Finger millet · Morphological · Antioxidants · Biochemical · AAS

INTRODUCTION

Our environment has a lot of heavy metals such as chromium, cadmium, cobalt, lead, mercury and copper etc. Interestingly, small amounts of these elements are actually necessary for good health, but large amounts of any of them may cause acute or chronic toxicity. The presence of heavy metals in the environment is of major concern because of their toxicity and threat to plant and animal life. Cobalt (Co) is a natural earth element presented in trace amount in soil and necessary for normal metabolic functions in plants, but at higher concentration it is toxic and may severely interfere with physiological and biochemical functions [1]. In excess concentration it caused a marked reduction in growth together with chlorosis and necrosis [2]. Many of these plant responses to heavy metals are as a result of the inhibition of enzymatic activity caused by the binding of heavy metal ions to sulfhydryl groups in the active sites of enzymes and by substitution of essential metals [3]. The activities of several enzymes are also disturbed [4]. In general, the average level of Co in the soil ranges 30-40 ppm [5] and above that it generates toxicity. Plant species vary in their sensitivity to Co. Soil type and soil chemistry greatly influences Co toxicity. One of the most important soil properties is soil acidity and more acidic the soil, the greater the potential for Co toxicity, at any concentration. During seed germination it causes ultra – structural changes & may cause inhibition in growth of plumule & radicals [6] and concentrations suppressed the seedlings growth and dry weight of the ragi seedlings [7].

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MATERIALS AND METHODS

Both control and experimental plants were allowed to grow in soil containing red, black and sand in the ratio of 1:1:1. After seven days the seedling of *Eleusine coracana* (L) Gaertn were treated with various concentrations of cobalt chloride such as 2, 4, 6, 8 & 10mM. After ten days of cobalt treatment various morphometric, pigmental, biochemical and enzymatic characters were analyzed.

The morphometric characters such as shoot length and root length are measured by scale and expressed in the leaf area was measured by conventional graphical method by drawing the outline of the leaf in a graph sheet and counting the small and big squares with in it and represented in cm^2. The fresh weight and dry weight of the seedlings was obtained using an electronic balance.

The pigmental characters such as chlorophyll a, chlorophyll b, total chlorophyll and carotenoids are measured by conventional method [8].

The biochemical characters such as total soluble sugars are measured by centrifugation after treating the tissue use tri-chloro acetic acid and anthrone as the indicator the total soluble sugars. The amino acid content was measured by homogenation of leaf tissue in ethanol and ninhydrine is the indicator of amino acid [9]. The protein content was measured by centrifugation leaf tissue in tri-chloro acetic acid, folin phenol with alkaline copper mixture [10]. The proline was estimated by centrifugation of leaf tissue in sulphosalicylic acid, glacial acetic acid and the proline was separated in the toluene [11].

The *In vivo* Nitrate Reductase (NR) activity was assayed according to Jaworski *et al* 1971[12] method with modification. Fresh leaf material (100mg) was incubated in scintillation vials containing 5 mL of incubation medium composed of, 100mM KH_2PO_4-KOH buffer pH 7.5, 200mM KNO_3, 1%(v/v) n-Propanol and 0.1%(v/v) Triton X 100. Incubation was carried out in dark for one hour at room temperature with occasional shaking. Aliquots of 0.5 mL was taken from the vials and analysed for nitrite after 1 hour incubation. To 0.5 mL distilled water, 1 mL of 3% sulphanilamide and 1 mL of N-1-N (Naphthyl ethylene-diamine dihydrochloride) were added in quick succession. Fifteen minutes were allowed for colour formation and absorbance was measured at 540 nm.

The nitrite was estimated with the help of a standard nitrite curve. The preparation of peroxidase and catalase enzyme extracts is, the leaves of experimental plants weighing about 1 g was ground in 5 mL of 100 Mm phosphate buffer (pH 6.0) and filtered through a three layered cheese cloth and spun at 3000 rpm for 30 minutes. The supernatant obtained was served as the source for crude enzymes such as peroxidise and catalase.

To assay peroxidise activity, the enzyme extract was added to pyrogallol which gets oxidized to a coloured derivative in the presence of hydrogen peroxide (1% (v/v). The amount of purpurogallin formed during the reaction was assayed spectroscopically [13]. To 2 mL of pyrogallol phosphate buffer (0.058 M pyrogallol dissolved in 0.1 M phosphate buffer pH 6.0), an aliquot of 0.1 mL of enzyme extract was added. Then, absorbance was set to zero at 420nm. To this, 0.5 mL of H_2O_2 (1% (v/v)) was added. Then, the content was thoroughly mixed and the absorbance was measured using systronics model no. 106 spectrophotometer. The difference in the absorbance at an interval of 20 seconds for a period of 3 minutes was measured. The peroxidise activity was expressed as moles of H_2O_2 reduced per unit enzyme per unit time.

To assay the catalase activity, 3 mL of phosphate buffer was added to 1 mL of H_2O_2 and 1 ml of enzyme extract [14]. The reaction mixture was incubated at 25ºC for 1 minute. The reaction was terminated by the addition of 1 mL of H_2SO_4. The reaction mixture was titrated against 0.01 N $KMNO_4$. The end point was the persistence of pink colour at least for 15 seconds. The catalase activity was expressed in micromoles H_2O_2 catalysed per unit time per mg protein.

The heavy metal accumulation of the experimental plants was analyzed at the end of the plant life. Cobalt concentrations in plants were analyzed using the method [15].

The plant sample as a whole was washed, dried in oven at 160ºC for 40 minutes and digested in a mixture of nitric acid and perchloric acid (10:1). Then the solution was centrifuged at 5000 rpm for 5 minutes and double filtered with Whatmann filter paper no.4 and the filtrate was analyzed for cobalt concentration by Atomic Absorption Spectrometry (Shimadzu Model AA – 6300), available in the Science Instrumentation Centre of Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi, Tamilnadu.

Statistical Analysis: For the morphometric characters the average value of ten independent samples and for the biochemical readings and enzymatic characters the average value of five were considered. The data were reported as mean ± SE and the percent activity was represented in the parentheses. Statistical analysis (One way ANOVA – Turkey test) was done using the statistical package, Origin – version 7.0.

RESULTS

The results showed that the morphometric characters such as root length, shoot length, leaf area, fresh weight and dry weight were decreased with increasing the concentration of cobalt chloride (Table 1). The levels of chlorophyll and carotenoids decreased but the level of anthocyanin increased (Table 2). Similarly the total soluble sugars and protein also showed a declining trend with increasing the concentration of cobalt chloride (Table 3). In contrary, the leaf nitrate, free amino acid and proline (Table 3) and the activities antioxidant enzymes such as peroxidase and catalase increased with the increase in the metal concentration. But nitrate reductase activity decreased (Table 4). The metal content of the treated and control seedlings of *Eleusine coracana* (L) Gaertn. was finally estimated by AAS study Table (5).

Table 1: Impact of various concentration of cobalt chloride on the morphometric characteristics of *Eleusine coracana* (L.) Gaertn.

Cobalt concentration (mM)	Shoot Length (cm)	Root Length(cm)	Leaf area (cm²)	Fresh weight (g)	Dry weight (g)
Control	17.16±0.545 (100)	9.46±0.202 (100)	4.33±0.145 (100)	0.25±0.030 (100)	0.12±0.01 (100)
2	15.26±0.497 (88.93)	7.933±0.202 a* (83.81)	4.033±0.346 a* (93.08)	0.21±0.014 a* (83.14)	0.10±0.014 a* (84.21)
4	13.46±0.317(78.45)	6.933±0.145 a*(73.24)	3.533±0.088 a*(81.54)	0.14±0.012 a*(55.86)	0.08±0.012 a*(65.79)
6	12.26±0.272(71.46)	6.433±0.088 a*(67.96)	9.85±0.263 a*(76.78)	0.10±0.008 a*(41.57)	0.07±0.008 a*(60.52)
8	11.66±0.145(67.97)	5.733±0.666 a*(60.57)	9.52±0.216 a*(74.21)	0.09±0.012 a*(37.67)	0.05±0.008 a*(44.74)
10	10.10±0.103(58.85)	4.766±0.033 a*(50.36)	8.02±0.174 a*(62.52)	0.063±0.008 a*(24.68)	0.04±0.005 a*(31.58)

Eleusine coracana (L.) Gaertn.

Values are an average of ten observations. Values in parenthesis are percentage activity with respect to control. Mean±SE a – 2mM to 10mM Concentrations compared with control, *Significant (P = 0.05 – Turkey test).

Table 2: Impact of various concentration of cobalt chloride on the photosynthetic pigments of *Eleusine coracana* (L.) Gaertn.

Cobalt concentration (mM)	Chlorophyll a (mg/g LFW)	Chlorophyll b (mg/g LFW)	Total chlorophyll (mg/g LFW)	Carotenoids (mg/g LFW)	Anthocyanin (mg/g LFW)
Control	16.49±0.079 (100)	13.39±0.352 (100)	31.22±0.147 (100)	0.22±0.007(100)	14.68±0.235(100)
2	16.44±0.146 a* (99.71)	09.63±0.231 a* (71.94)	26.75±0.354 a* (85.68)	0.18±0.005 a*(81.19)	16.03±0.214 a*(109.2)
4	15.25±0.154 a*(92.48)	08.70±0.616 a* (64.96)	24.29±0.741 a* (77.79)	0.16±0.003 a*(73.54)	16.65±0.351 a*(113.4)
6	13.61±0.475 a*(82.56)	06.67±0.283 a*(64.53)	20.95±0.447 a*(67.12)	0.13±0.002 a*(60.68)	18.40±0.287 a*(125.3)
8	12.54±0.281 a*(76.06)	04.57±0.636 a* (44.23)	17.78±0.824 a*(56.97)	0.10±0.005 a* (48.36)	19.71±0.820 a*(134.3)
10	10.61±0.296 a*(64.35)	04.21±0.258 a* (40.78)	14.01±0.469 a*(46.44)	0.09±0.002 a*(40.20)	20.29±0.661 a*(138.2)

Values are an average of ten observations. Values in parenthesis are percentage activity with respect to control. Mean±SE a – 2mM to 10mM Concentrations compared with control, *Significant (P = 0.05 – Turkey test).

Table 3: Impact of various concentration of cobalt chloride on the biochemical characteristics of *Eleusine coracana* (L.) Gaertn.

Cobalt concentration (mM)	Total soluble sugar (mg/g LFW)	Total soluble protein (mg/g LFW)	Amino acid (µ Mole/g LFW)	Proline (mg/g LFW)	Leaf nitrate (mg/g LFW)
Control	5.52±0.178 (100)	1.22±0.037(100)	16.36±0.306(100)	2.91±0.036(100)	0.11±0.001(100)
2	4.923±0.031 a*(89.08)	1.06±0.027 a*(87.32)	18.41±0.573 a*(112.6)	3.62±0.074 a*(124.2)	0.15±0.004 a*(140)
4	3.78±0.051 a*(68.40)	0.91±0.035 a*(74.64)	20.65±0.430 a*(126.2)	4.30±0.050 a*(147.6)	0.17±0.002 a*(160.6)
6	2.91±0.028 a*(52.65)	0.79±0.013 a*(65.24)	26.25±0.473 a*(160.4)	5.14±0.042 a*(176.4)	0.22±0.002 a*(204.8)
8	2.25±0.053 a*(40.83)	0.71±0.018 a*(58.69)	36.61±0.786 a*(210.4)	5.61±0.034 a*(192.5)	0.27±0.001 a*(247.7)
10	1.53±0.103 a*(27.80)	0.64±0.013 a*(52.56)	49.61±0.620 a*(297.6)	5.78±0.042 a*(200.4)	0..34±0.003 a*(306)

Values are an average of ten observations. Values in parenthesis are percentage activity with respect to control. Mean±SE a – 2mM to 10mM Concentrations compared with control, *Significant (P = 0.05 – Turkey test).

Table 4: Impact of various concentration of cobalt chloride on the enzymes activity of *Eleusine coracana* (L.) Gaertn.

Cobalt concentration (mM)	NR Activity (µ Mole/g LFW)	Peroxidase (µ Mole/g LFW)	Catalase (µ Mole/g LFW)
Control	10.77±0.069 (100)	6.88±0.030 (100)	1.55±0.058 (100)
2	7.24±0.145 a* (67.53)	7.00±0.016 a* (101.8)	2.15±0.058 a* (138.6)
4	6.52±0.154 a* (60.88)	7.19±0.029 a* (104.5)	2.86±0.117 a* (187.1)
6	5.91±0.097 a* (54.15)	7.99±0.020 a* (116.1)	3.66±0.117 a* (224.3)
8	5.38±0.048 a* (50.26)	8.68±0.039 a* (126.2)	3.73±0.058 a* (247.1)
10	4.69±0.042 a* (44.56)	10.01±0.067 a* (144.9)	4.22±0.080 a* (271.4)

Table 5: AAS study indicating the content of cobalt in *Eleusine coracana* (L.) Gaertn.

SAMPLE	AAS in ppm
SAMPLE 1 (Control)	0.8818ppm
SAMPLE 2 (2 mM)	3.2406ppm
SAMPLE 3 (4 mM)	4.1155ppm
SAMPLE 4 (6 mM)	4.7864ppm
SAMPLE 5 (8 mM)	5.5056ppm
SAMPLE 6 (10 mM)	6.7484ppm

Values are an average of ten observations. Values in parenthesis are percentage activity with respect to control. Mean±SE a – 2mM to 10mM Concentrations compared with control, *Significant (P = 0.05- Turkey test).

DISCUSSION

Heavy metal is known to enhance the level of the enzyme chlorophyllase that brings about the degradation of the chlorophyll [16]. The reduction in sugar contents may be attributed to reduction in chlorophyll contents of the leaf and also a decline in protein. This change might have already affected the photosynthetic activity of the plant and hence the reduction in contents [17].

Accumulation of proline has been frequently used as biochemical marker for water stress in plants [18]. In stress condition the inhibition of growth of cells, leaves and the whole plant were accompanied by an accumulation of nitrate in plant tissue particularly in leaves [19]. The leaf nitrate content was found to be more in cobalt treated plants paralleling with the reduction in nitrate reductase activity. The peroxidase activity was found to be increased with increasing the concentration of the cobalt chloride and cause a major impact on the chlorophyll degradation. Heavy metals are well known, to generate a large quantity of reactive oxygen species (ROS) in plants that may oxidize protein, lipids and nucleic acids resulting in the abnormalities at the level of the cell [20].

To maintain metabolic functions under stress conditions, the balance between generation and degradation of ROS is required; for this purpose plants have well equipped antioxidant system that include antioxidant enzymes (superoxide dismutase, catalase, peroxidase and glutathione reductase) and non- enzymatic low molecular weight antioxidants (glutathione, proline, carotenoids, tocopherols etc.) [21]. An increase in the amino acid and proline content after match, sugar industrial effluent treatment has already been reported [22][23]. Catalase is an antioxidant and scavenging enzyme, found to be increased with the increasing concentration of cobalt chloride. Both the catalase and peroxidase catalyse the degradation of H2O2, which is a natural metabolite and also toxic to plants [24].

CONCLUSION

The results of present investigation inferred from the comparison of the values given in parenthesis of treated plants with control reveals that cobalt chloride has seriously affected the finger millet plants.

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