



Growth and biochemical contents of Cowpea (*Vigna unguiculata* L.) on the application of zinc

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ABSTRACT

The present investigation deals with the effect of zinc on the morphological and biochemical parameters of Cowpea (*Vigna unguiculata* L.) under laboratory condition. The Cowpea plants are grown in petriplates lined with filter paper treated with various concentrations (control, 10, 25, 50, 75, 100, 150 and 200 ppm) of zinc. The experiments were replicated five times. The various morphological parameters such as germination percentage, shoot length, root length, root nodules, total leaf area, fresh weight and dry weight of the plants were measured. Similarly the biochemical contents such as chlorophyll, carotenoid, protein and Sugar was analyzed. The results indicated that the various morphological and biochemical parameters increased up to 50 ppm and decreased at high levels of zinc.

Keywords: *Vigna unguiculata*; Cowpea; Zinc

1. INTRODUCTION

Environmental pollution constitutes a great health hazard to human, animals and plants with local, regional and global implications (Irshad *et al.*, 1997). Pollution has adverse effects

on land, water or air and its biotic and abiotic components. Water pollution may result from municipal, agricultural or industrial wastes containing organic and inorganic chemical substances, dissolved or suspended solids (Moeller, 2004). Increasing population and consequential increase in food demand had required intensive year round food production. Hence, irrigation of arable land and crops from water bodies is very important to ensuring food satisfactoriness. Increasingly, effluent-laden water bodies have become sources for crop irrigation systems sometimes preferred for added nutrients from municipal wastes, distillery or agro-based industries (Shrestha and Niroula, 2003). The problem of the toxic heavy metals had recently been given much attention, especially after the reorganization of soil pollution and the widespread industrialization and urbanization.

Heavy metal contamination of the soil is the main environmental problem in many countries. Heavy metals are basically characterized by their toxicity, persistence and difficulty to be removed. Some of them participate in a number of biochemical reactions in living beings, raising the attention regarding the toxic effects upon the plants. The toxicity of heavy metals is a problem for ecological, evolutionary and environmental reasons (Nagajyoti *et al.*, 2008). High concentrations of metals exert a negative influence on the development of plants, their use of nutrient and metabolism. The heavy metals can cause a major ecological crisis since they are non-degradable and often accumulate in plant parts, biologically magnified through trophic levels and causes deleterious effects on plants and animals.

Zinc is one of the first micronutrients recognized as essential for plants that transported to plant root surface through diffusion (Maqsood *et al.*, 2009). Zn is a micronutrient and in case of its severe deficiency the symptoms may last throughout the entire crop season (Asad and Rafique, 2000). Zn deficient plant also appears to be stunted (Torun *et al.*, 2001) as a result approximately 2 billion people suffer from Zn deficiency all over the world (Asad and Rafique, 2002).

The grain yield can be improved by addition of Zn fertilization (Maqsood *et al.*, 2009). Bora and Hazarika, (1997) reported highest stover yield (2770 kg ha^{-1}) with Zn and almost the same trend of seed yield. The seed yield can be improved by addition of Zn fertilization. Chen and Aviad, (1990) found that application of Zn alongwith other micronutrients improved soil organic matter and resulted in increasing mustard yields. Kutuk *et al.*, (2000) also suggested that the application of Zn has become necessary for improved crop yields. Mandal and Sinha, (2004) recommended application of ZnSO_4 at the rate of 20 kg ha^{-1} for oilseeds including mustard. Moniruzzaman *et al.*, (2008) applied zinc at the concentrations of 0, 2.5, 5.0 and 7.5 kg ha^{-1} and suggested 8 kg Zn ha^{-1} for *Brassica* species. Although reports have been focused on the toxic effects of nickel on plants, more knowledge of its toxicity is required and the detailed mechanisms involved in its toxicity has to be investigated. So the present study aims to bring the facts of Zinc toxicity on cow pea with special reference to growth, chlorophyll, protein and sugar contents.

2. MATERIALS AND METHODS

The Cowpea (*Vigna unguiculata* L.) (Photo 1 and 2) seeds were obtained from Tamilnadu Agricultural University, Vamban, Pudukkottai District, Tamil Nadu. The seeds are uniform size, colour was selected for the experimental purpose. Zinc sulphate was used as the source of preparation of stock solution of Zinc.



Photo 1. *Vigna unguiculata*.



Photo 2. *Vigna unguiculata*.

From this stock solution different concentrations (Control, 10, 25, 50, 75, 100, 150, 200 and 300 ppm) of Zinc solution were prepared freshly at the time of experiments. The selected cow pea seeds were sown in the petriplates lined with filter paper. The seeds irrigated with normal tap water was maintained as the control.

Shoot length and root length

Five plants were randomly selected for recorded the shoot length and root length of experimental plants. They were measured by using centimetre scale.

Root nodules

Five plants from each plastic cup with intact roots were removed with the help of digging fork. The root nodules were carefully separated from the soil by gently pinching and washing the soil particles. The following characters were recorded.

Total leaf area (Kalra and Dhiman, 1977)

Five plant samples were collected at 7th day sampling plants and the length and breadth of the leaf samples were measured and recorded. The total leaf area was calculated by using the Kemp's constant.

$$\text{Total leaf area} = L \times B \times K$$

where:

L - length, B - breadth and K - Kemp's constant (for dicot - 0.66).

Fresh weight and dry weight

Five plant samples were randomly selected at 7th day plants. Their fresh weight was taken by using an electrical single pan balance. The fresh plant materials were kept in a hot air oven at 80 °C for 24 hrs. and then their dry weight were also determined.

Biochemical Analyses

The photosynthetic pigments such as chlorophyll a, b, total chlorophyll and carotenoid and the biochemical contents such as protein, and sugars (reducing, non-reducing and total sugars) were analysed in the treatment plants. The test plants were randomly collected at 7th day of plants

Chlorophyll (Arnon, 1949)

Five hundred mg of fresh leaf material was ground with a mortar and pestle with 10 mL of 80 per cent acetone. The homogenate was centrifuged at 800 rpm for 15 min. The supernatant was saved and the residue was re-extracted with 10 mL of 80 per cent acetone. The supernatant was saved and the absorbance values were read at 645 and 663 nm in a UV-spectrophotometer. The chlorophyll *a*, chlorophyll *b* and total chlorophyll contents were estimated and expressed in mg g⁻¹ fresh weight basis.

$$\text{Chlorophyll 'a'} = (0.0127) \times (\text{O.D } 663) - (0.00269) \times (\text{O.D } 645)$$

$$\text{Chlorophyll 'b'} = (0.0229) \times (\text{O.D } 645) - (0.00488) \times (\text{O.D } 663)$$

$$\text{Total chlorophyll} = (0.0202) \times (\text{O.D } 645) + (0.00802) \times (\text{O.D } 663)$$

Carotenoid (Kirk and Allen, 1965)

The same plant extract used for chlorophyll estimation was used for carotenoid estimation. The acetone extract was read at 480 nm in a UV-spectrophotometer. The carotenoid content was calculated by using the following formula and it is also expressed in mg g⁻¹ fresh weight basis.

$$\text{Carotenoid} = (\text{O.D } 480) - (0.114) \times (\text{O.D } 663) - (0.638) \times (\text{O.D } 645)$$

Estimation of protein (Lowry *et al.*, 1951)

Extraction

Five hundred mg of plant materials (root, stem and leaf) were weighed and macerated in a pestle and mortar with 10 mL of 20 per cent trichloroacetic acid. The homogenate was centrifuged for 15 min at 600 g. The supernatant was discarded. To the pellet, 5 mL of 0.1 N NaOH was added and centrifuged for 5 min. The supernatant was saved and made up to 10 mL of 0.1 N NaOH. This extract was used for protein estimation.

Estimation

One mL of the extract was taken in a 10 mL test tube and 5 mL of reagent 'C' was added. The solution was mixed and kept in darkness for 10 min. Later, 0.5 mL of Folin-phenol reagent was added and the mixture was kept in dark for 30 min. The sample was read at 660 nm in a UV-spectrophotometer.

Preparation of reagents

Reagent A: 0.4 g of sodium hydroxide was dissolved in 100 mL of distilled water. To this solution, 2 g of sodium carbonate was added.

Reagent B: One per cent of copper sulphate was mixed with equal volume of 2 per cent sodium potassium tartarate.

Reagent C: Fifty mL of reagent A and one mL of reagent B were taken and mixed freshly at the time of experiment.

Folin-phenol reagent: One mL of Folin-phenol reagent was diluted with 2 mL of distilled water.

Estimation of sugars (Nelson, 1944)

Extraction

Five hundred mg of plant materials were weighed and macerated in a pestle and mortar with 10 mL of 80 per cent ethanol. The homogenate was centrifuged for 10 min at 800 g. The supernatant was saved. Then, the ethanol was evaporated in water bath at 50 °C. The net

content was made up to 20 mL with distilled water and the extract was used for the estimation of reducing sugar.

Estimation

One mL of extract was taken in a 25 mL marked test tube. One mL of reagent 'C' was added. Then, the mixture was heated for 20 min at 100 °C in a boiling water bath, cooled and 1 mL of arseno-molybdate reagents was added. The solution was thoroughly mixed and diluted to 25 mL with distilled water. The sample was read at 520 nm in a UV-spectrophotometer.

Preparation of reagents

Reagent A: Twenty five gram of anhydrous sodium carbonate, 25 g of sodium potassium tartarate, 20 g of sodium bicarbonate and 200 g of anhydrous sodium sulphate were dissolved in 800 mL of distilled water and made up to 1000 mL. Then, it was filtered and stored in a glass stoppered brown bottle.

Reagent B: Fifteen per cent copper sulphate containing 1 or 2 drops of concentrated sulphuric acid.

Reagent C: Fifty mL of reagent A and one mL of reagent B were mixed and it was prepared freshly at the time of experiment.

Arsenomolybdate reagent: To 450 mL of distilled water, 25 g of ammonium molybdate, 21 mL of concentrated sulphuric acid were added and 3 g of sodium arsenate was dissolved in 25 mL of distilled water. The mixture was kept in water both at 37 °C for 24 to 48 hr. The reagent was stored in a glass stoppered brown bottle.

3. RESULTS AND DISCUSSION

Zinc is essential micronutrients for proteins production in plants; also zinc is main composition of ribosome and is essential for their development. Amino acids accumulated in plant tissues and protein synthesis decline by zinc deficit. One of the sites of protein synthesis is pollen tube that amount of zinc in there tip is 150 micrograms per gram of dry matter. In addition zinc will contribute on the pollination by impact on pollen tube formation (Marschner, 1995).

Increasing seed concentration of Zn by soil and/or foliar applications of zinc also brings several agronomic benefits for crop production. Applying zinc to plants grown under potentially zinc-deficient soils is effective in reducing uptake and accumulation of phosphorus (and thus phytate) in plants. This agronomic side effect of zinc fertilization may result in better bioavailability of zinc in the human digestive system. In addition, seedlings from seeds containing high zinc have better ability to withstand adverse environmental conditions. These benefits are discussed in detail below (Cakmak, 2008).

Seed has been one of the most important agriculture inputs. Seed germination and growth are of vital importance for continuity of plant life. Seed germination is defined as the resumption of metabolic process. The growth of an embryo begins with the rupture of the seed coat and the emergence of the young plant. The first process occurring in germination viz., water uptake involves both imbibitions and osmosis.

After, the rate of uptake of water is reduced and germination proceeds. Subsequently, the growth and the development of the seedlings also begin. The time between the sowing of seed and establishment of that seedling is considered to be the crucial period of any plant. The seed when exposed to a wide range of environmental stress, the germination performance and the establishment of seedlings gets adversely affected (Lalitha *et.al*, 1999).

Germination of seeds and growth of nascent seedlings are dependent process accountable for initial establishment of plant. Since these growth phases are sensitive to environmental stresses, they are well suited for phytotoxicity assessment (Jetly and Srivastava, 1995), Heavy metal pollution of water is major environment problem facing the modern world. Heavy metals are those chemicals elements having specific weights more than 5 g cm^3 (Sharma and Agarwal, 2005). There are 40 elements that fall into this category.

In the present study cow pea seeds were treated with different concentrations of zinc solutions for testing the growth promoting efficiency. Ten day old cowpea seedlings were used to analyze various morphological parameters such as germination percentage, root length, shoot length, number of root nodules, total leaf area, fresh weight and dry weight (Table 1 and 2).

All the morphological parameters increased up to 50 ppm concentrations of zinc when compared with control. Similarly the morphological parameters are decreased gradually with the increase of zinc concentrations. Similar decrease in plant height was observed by Sharma and Sharma, 1993, Moustakas *et al.* 1994 and Vijayarengan, 2012.

The improvement in the growth efficiency of plant organ might also be due to beneficial effects of zinc treatments on the physiological activities and other enzyme reaction in the transformation of carbohydrates and activities of hexokinase of plants which were responsible in improving the growth of plant and its component organs ultimately influencing the relative development of plant parts and their growth efficiency (Vijayarengan and Mahalakshmi., 2013). Similarly the biochemicals such as chlorophyll *a*, chlorophyll *b*, Total chlorophyll, Carotenoid, Protein and Sugar content is also increased upto 50 ppm zinc concentrations and gradually decreased at the increase of zinc concentrations (Table 3).

Chlorophyll is an integral component of plant pigments and plays a vital role in the process of photosynthesis. The reduction in chlorophyll content was observed from 5 to 200 mg/l chromium concentrations. The decrease in chlorophyll content may be due to the interference of Cr with pigment metabolism. Cr absorbed by plants caused inhibition of important enzymes in chlorophyll biosynthesis. Impaired aminolaevulinic acid dehydrogenase activity and photochlorophyllide reductase leading to reduced photosynthetic pigments has been observed in chromium treated *Nymphaea alba* (Vajpayee *et al.*, 1986).

The decrease in chlorophyll content may be due to destabilization and degradation of proteins of the peripheral part. The inactivation of enzymes involved in the chlorophyll content in most plants under chromium stress was reported (Shanker.,*et al.*, 2005). Similar results were noted in the earlier studies of Balashouri and Prameeladevi, 1995; Bonnet *et al.*, 2000; Sharma *et al.*, 2009 and Umebese and Motajo, 2008 in various crops. Zinc at high levels may inhibit the root growth directly by inhibition of cell division or cell elongation or Combination of both, resulting in the limited exploration of the soil volume for uptake and translocation of nutrients and water and induced mineral deficiency (Foy, 1988). The results of the present study also confirmed these views.

Table 1. Effect of different concentrations of Zinc (ppm) on growth parameters of cow pea on 10th day plant.

Zinc conc. (ppm)	Germination percentage	Shoot length (cm/plant)	Root length (cm/plant)	Root nodules
Control	95.00 ±4.75	6.55 ±0.675	4.70 ±0.285	15.30 ±2.16
10	96.00 ±3.90	8.13 ±0.456	5.32 ±0.216	17.21 ±1.61
25	98.00 ±3.10	9.10 ±0.355	5.54 ±0.177	19.73 ±1.33
50	99.00 ±2.55	12.36 ±0.318	6.02 ±0.151	20.31 ±0.968
75	72.00 ±2.10	5.82 ±0.141	3.02 ±0.051	12.38 ±0.469
100	63.00 ±1.15	4.10 ±0.105	2.90 ±0.045	8.54 ±0.140
150	62.00 ±1.12	3.00 ±0.141	1.85 ±0.041	7.65 ±0.121
200	60.00 ±1.12	2.87 ±0.045	1.70 ±0.041	5.32 ±0.120

± Standard deviation

Table 2. Effect of different concentrations of Zinc (ppm) on growth parameters of cow pea on 10th day plant.

Zinc conc. (ppm)	Total Leaf area (cm ² /leaves)	Fresh weight (mg/g fr. wt.)	Dry weight (mg/g dry wt.)
Control	3.02 ±0.301	2.87 ±0.293	0.60 ±0.11
10	3.65 ±0.282	3.18 ±0.209	0.80 ±0.09
25	4.02 ±0.251	4.27 ±0.114	1.02 ±0.05
50	5.85 ±0.192	5.63 ±0.081	1.70 ±0.03
75	2.46 ±0.123	2.80 ±0.041	0.59 ±0.02
100	2.52 ±0.026	2.36 ±0.018	0.50 ±0.02
150	1.50 ±0.020	1.00 ±0.041	0.43 ±0.02
200	1.23 ±0.020	0.80 ±0.041	0.34 ±0.02

± Standard deviation

Table 3. Effect of different concentrations of Zinc (ppm) on Biochemical content of cow pea on 10th day plant.

Zinc Conc. (ppm)	Chl 'a' (mg/g fr. wt.)	Chl 'b' (mg/g fr. wt.)	Total Chlorophyll	Carotinoid (mg/g fr. wt.)	Protein (mg/g fr. wt.)	Total sugar (mg/g fr. wt.)
Control	2.76 ±0.143	1.71 ±0.090	4.47 ±0.23	2.43 ±0.101	2.11 ±0.145	1.83 ±0.021
10	2.82 ±0.101	2.28 ±0.064	5.10 ±0.165	2.86 ±0.093	2.34 ±0.117	1.91 ±0.030
25	3.27 ±0.063	3.07 ±0.048	6.34 ±0.112	3.50 ±0.075	3.63 ±0.081	2.94 ±0.047
50	4.91 ±0.045	3.44 ±0.022	8.35 ±0.067	3.91 ±0.045	3.88 ±0.054	2.75 ±0.067
75	2.58 ±0.029	1.38 ±0.019	3.96 ±0.048	2.06 ±0.033	1.92 ±0.046	1.29 ±0.089
100	1.29 ±0.014	1.05 ±0.007	2.34 ±0.022	1.54 ±0.027	1.53 ±0.026	1.71 ±0.135
150	0.73 ±0.014	0.56 ±0.063	1.29 ±0.063	1.25 ±0.063	1.00 ±0.063	0.98 ±0.063
200	0.65 ±0.063	0.45 ±0.063	1.10 ±0.063	1.04 ±0.063	0.78 ±0.063	0.75 ±0.063

± Standard deviation

4. CONCLUSION

The present investigation concluded finally, zinc in low level increase the plants growth and biochemical contents. Similarly at higher concentrations it may reduce the growth as well as biochemical metabolism. So the application of zinc at low level proves to be a micronutrient in crops. In contrary at higher concentration may act as a heavy metal.

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