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RESEARCH ARTICLE

CHANGES IN THE BIOCHEMICAL CONSTITUENTS OF *VIGNA MUNGO* UNDER ZINC EXPOSURE

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ABSTRACT

In the present study *Vigna mungo* plants were raised under different concentrations of zinc (5, 10, 25, 50 & 100 mgl^{-1}). One set of seeds were irrigated with distilled water which served as control (0 mgl^{-1}). *Vigna mungo* seeds were equispacealy placed in Petri plates lined with filter paper. They were irrigated periodically with various concentrations (5, 10, 25, 50 & 100 mgl^{-1}). Five replicates were maintained for each concentrations including control. The number of seeds germinated were counted and recorded daily. On the 10th day after sowing five seedlings were taken in random to record various seedling growth parameters like shoot length, root length and dry weight of seedlings. The seedlings were used for the analysis of various biochemical constituents like photosynthetic pigments, protein, total sugars and amino acids. There was a gradual decline in all the growth parameters and biochemical constituents with progressive increase in zinc concentration.

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INTRODUCTION

Industrialization has been considered as the fastest route for the economic development of a country. It also serves as a major indicator for the quality of environment of the country. Industries are of great concern as they discharge huge amount of wastes into the neighbouring water bodies making them undesirable for irrigation. All industrial wastes affect the normal life of the aquatic organisms as well as the growth and productivity of crops when utilized for irrigation. In aquatic environment metals can be termed as "conservative pollutants", which are added to the environment and cannot be broken into harmless substances by bacterial action. Among the various kinds of pollution heavy metals deserves a special attention. Although some metals are essential for plant growth in small quantities, it is quite obvious from many researches that heavy metals produce adverse effect on growth and productivity of plants (Deram *et al*. 2000). Zinc is a component of many enzymes, it influences photosynthesis, it regulates the carbohydrate metabolism and proteins. It is involved in reducing nitrates. Although zinc is a micro nutrient with important functions in the plant life, it becomes toxic at high concentration (Anisorastratu *et al*. 2010). An attempt has been made in the present investigation to explore and analyze the affect of zinc on germination, seedling growth and biochemical constituents of black gram *(Vigna mungo)*.

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MATERIALS AND METHODS

Seed materials

Seed The experimental plant, the cowpea (*Vigna mungo* (L.) Walp.) belongs to the family Fabaceae is one of the important pulses of India. Seeds used in the experiments were obtained from the Pulses Division, Agriculture Research Institute, Thindivanam, Tamil Nadu. Seeds with uniform size, colour and weight were chosen for experimental purpose.

Germination studies

The germination studies were carried out according to the Top paper method recommended by the International seed testing Association (1979). Various concentrations of zinc sulphate (0, 5, 10, 25, 50 & 100 mgl^{-1}) were prepared by using distilled water. Healthy seeds of black gram were selected and surface sterilized with 0.1% mercuric chloride solution for two minutes. The seeds were thoroughly washed under running water. Twentyfive seeds were evenly placed in each Petri plate lined with filter paper. They were irrigated daily with respective concentration of zinc sulphate solution. One set (0 mgl^{-1}) was irrigated with distilled water (control). Five replicates were maintained for each concentration as well as control. The percentage of seeds germinated was recorded daily. The various seed germination characteristics like rate of germination, germination index (Carley and Watson, 1968) vigour index (Abdul Baki and Anderson, 1978), tolerance

index (Turner and Marshal, 1972) a seedling growth parameters like root length, shoot length and dry weight of seedlings were recorded on the ninth day after sowing.

Biochemical analysis

Chlorophyll 'a', chlorophyll 'b', total chlorophyll, Protein, total sugars and amino contents in plant samples were estimated by the following methods

Estimation of chlorophyll

Hundred milligram of fresh leaf was ground in a mortar and pestle with 20 ml of 80% acetone. The homogenate was centrifuged at 3000 rpm for 15 minutes. The supernatant was saved. The pellet was reextracted with 5ml of 80% acetone each time, until it become colourless. All the supernatants were pooled and utilized for chlorophyll determination. Absorbance was read at 645nm and 663nm in spectrophotometer. The chlorophyll content was measured by using the formula given by Arnon (1949).

Estimation of protein

Fresh tissue weighing 0.5 g was macerated in 20 per cent trichloroacetic acid using mortar and pestle. The homogenate was then centrifuged at 600 rpm for 30 min and the supernatant was discarded. Five ml of 0.1 N NaOH was added to the pellet and it was centrifuged for 30 min. The supernatant was saved for the estimation of protein. To 0.5 ml of the extract, 5 ml of copper reagent 'C' was added (Reagent C: mixture of reagents A and B in the 50:1 ratio; Reagent A: 2 per cent Na_2CO_3 in 0.1 N NaOH; Reagent B: equal volume of 1 per cent $CuSO_4$ and 2 per cent sodium potassium tartrate). The tubes were shaken well and allowed to stand in dark for 10 min at room temperature, 0.5 ml of properly diluted Folin-Ciocalteau reagent was added to the solution and mixed thoroughly. The absorbance was read at 500 nm in a spectrophotometer against an appropriate blank. Bovin serum albumin was used as the standard (Lowry *et al*., 1951).

Estimation of total sugars

Plant samples were treated with 80 per cent boiling ethanol for taking extractions (5 ml extract representing 1 g of tissue). Five readings for each sample were taken. One ml of ethanol extract taken in the test tubes was evaporated in a water bath. To the residue, 1 ml of distilled water and 1 ml of 1 N sulphuric acid were added and ^{o}C for 30 min. The solution was neutralised incubated at 49 with 1 N sodium hydroxide using methyl red indicator. One ml of Nelson's reagent was added to each test tube prepared by mixing reagent A and reagent B in 25:1 ratio (Reagent A: 25 g sodium carbonate, 25 g sodium potassium tartarate, 20 g sodium bicarbonate and 200 g anhydrous sodium sulphate in 1000 ml; Reagent B: 15 g cupric sulphate in 100 ml of distilled water with 2 drops of concentrated sulphuric acid). The test tubes were heated for 20 min in a boiling water bath, cooled and 1 ml of arsenomolybdate reagent (25 g ammonium molybdate, 21 ml concentrated sulphuric acid, 5 g sodium arsenate dissolved in 475 ml of distilled water and incubated at 37 ^{o}C in a water bath for 48 h) was added. The solution was° thoroughly mixed and diluted to 25 ml and measured at 495 nm in a spectrophotometer. The reducing sugar contents of unknown samples were calculated from glucose standard (Nelson, 1944).

Estimation of total free amino acids

One ml ethanol extract was taken in 25 ml test tubes and neutralized with 0.1 N sodium hydroxide using methyl red indicator. One ml of ninhydrin reagent was added (800 mg stannous chloride in 500 ml citrate buffer, pH 5.0, 20 g ninhydrin in 500 ml methyl cellosolve; both solutions were mixed). The contents were boiled in a water bath for 20 min, 5 ml of diluent solution (distilled water and npropanol mixed in equal volume) was added, cooled and diluted to 25 ml with distilled water. The absorbance was measured at 570 nm in a spectrophotometer. The standard graph was prepared using leucine (Moore and Stein, 1948).

RESULTS AND DISCUSSION

The experiment was conducted to investigate the Changes in the biochemical constituents of *Vigna mungo* under Zinc exposure and different concentrations of zinc sulphate (0, 5, 10, 25, 50 & 100 mgl^{-1}).

Table 1. Effect of Zinc on the germination characteristics of black gram (*Vigna mungo* (L) Hepper)

Concentration (mgl^{-1})	Germination Rate (%)	Germination Index	Vigour Index	Tolerance Index
0	99	825.00	1150.42	-
5	100 (+1.01)	840.00 (+1.82)	1204.16 (+4.67)	0.99
10	95 (-4.04)	756.00 (-8.36)	1031.62 (-10.33)	0.85
25	90 (-9.09)	672.00 (-18.55)	926.38 (-19.47)	0.72
50	75 (-24.24)	585.00 (-29.09)	740.28 (-35.65)	0.66
100	60 (-39.39)	465.00 (-43.64)	612.02 (-46.80)	0.52

0 mgl^{-1} – Control; +,- Percentage of reduction over control values are given in parentheses.

Table 2. Effect of Zinc on the seedling growth parameters of black gram (*Vigna mungo* (L) Hepper)

Concentration (mgl^{-1})	Root length (cm plant $^{-1}$)	Shoot length (cm plant $^{-1}$)	Number lateral roots (plant $^{-1}$)	Dry weight of seedling (g plant $^{-1}$)
0	5.78	13.56	15.33	0.68
5	6.02 (+4.15)	14.15 (+4.35)	17.46 (+13.89)	0.725 (+6.62)
10	5.50 (-4.84)	11.77 (-13.20)	13.32 (-13.11)	0.605 (-11.03)
25	4.25 (-26.47)	10.16 (-25.07)	10.52 (-31.38)	0.532 (-21.76)
50	3.81 (-34.08)	9.62 (-29.06)	8.75 (-42.92)	0.412 (-39.41)
100	21.78 (-51.90)	8.50 (-37.32)	6.08 (-60.34)	0.315 (-53.68)

0 mgl^{-1} – Control; +,- Percentage of reduction over control values are given in parentheses.

Germination studies

According to the results, the rate of germination, germination index, vigour index and tolerance index are furnished in Table-1. The results indicate that at the lower concentrations

Table 3. Effect of Zinc on the biochemical constituents (mgl^{-1} dr. wt.) of black gram (*Vigna mungo* (L) Hepper)

Concentration (mgl^{-1})	Chlorophyll 'a'	Chlorophyll 'b'	Protein	Total Sugars	Amino acids
0	0.995	0.752	15.75	8.95	9.81
5	1.015 (+2.010)	0.815 (+8.378)	16.75 (+6.35)	10.56 (+17.99)	10.36 (+5.61)
10	0.870 (-12.563)	0.695 (-7.580)	14.186 (-9.930)	7.62 (-14.86)	8.68 (-11.52)
25	0.758 (-23.819)	0.512 (-31.915)	12.786 (-18.819)	6.50 (-27.37)	6.21 (-36.70)
50	0.562 (-43.518)	0.419 (-44.282)	11.126 (-29.359)	5.15 (-42.46)	5.75 (-41.39)
100	0.450 (-54.774)	0.340 (-54.787)	9.012 (-42.781)	4.38 (-51.06)	4.44 (-54.74)

0 mgl^{-1} – Control; +,- Percentage of reduction over control values are given in parentheses.

(5 mgl^{-1}) zinc induced all these parameters, but at the concentrations above 5 mgl^{-1} all the parameters showed a gradual decline. The germination study was conducted in the laboratory to find out the effect of zinc on seed germination, seedling growth, fresh weight and dry weight of black gram seedlings. In addition to that, biochemical analyses were also conducted in tolerant and susceptible varieties of Black gram under zinc concentration. It coincides with the reports of Saravanan *et al*. (2001), Shafiq and Iqbal (2005) and Imran *et al*. (2007).

Morphological parameters

The seedling growth parameters such as root and shoot length, number of lateral roots and dry weight of seedlings declined with progressive increase in zinc concentration when compared with control (Table 2). Similar reduction in seedling growth was also reported by Sidharthan and Lakshmanachary (1994), Kabir *et al*. (2008), Vijayaragavan *et al*. (2011) and Sofi Imtiyaz *et al*. (2014).

Biochemical studies

The results of biochemical constituents such as chlorophyll *a*, chlorophyll *b,* protein, total sugars and amino acids showed a slight increase at low concentration of zinc (5 mgl^{-1}) and it showed a simultaneous decline with increase in metal concentration. The results are inconsonance with the findings of Hsu and Kao, 2003, Qian *et al*. (2009) and Liantao Liu *et al*. (2014). The result of present study reveals that zinc elicited toxic effects on the germination characteristics, seedling growth and biochemical constituents of black gram. The lower concentration (5 mgl^{-1}) of zinc induced growth while higher concentration above 5 mgl^{-1} drastically inhibited the overall growth of the crop.

Conclusion

The present investigation deals with the zinc induced changes on growth, biochemicals of *Vigna mungo* plants. Zinc treatment at all levels tested decreased the various growth parameters such as length of the root and shoot, dry weight of seedlings; biochemical constituents (pigments, protein, sugars and amino acids contents of leaves) of *Vigna mungo* plants. From the present investigation it was concluded that the 5 mgl^{-1} level of zinc in the soil was beneficial for the growth of *Vigna mungo* plants. The level of zinc in the soil above 10 mgl^{-1} proved to be toxic. The results indicated that the 5 mgl^{-1} zinc level can be applied for increasing the growth and yield of *Vigna mungo* plants.

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