IMPACT: International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS)
ISSN (P): 2347–4580; ISSN (E): 2321–8851
Vol. 7, Issue 10, Oct 2019, 13–24

GERMINATING ABILITY, PHOTOSYNTHETIC ACTIVITY AND BIOCHEMICAL CHANGES OF COWPEA (*VIGNA UNGUICULATA* L. WALP) UNDER ZINC APPLICATION

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Received: **10 Oct 2020** **Accepted:** **15 Oct 2020** **Published:** **31 Oct 2020**

ABSTRACT

Effect of different concentrations of Zinc on, germination studies, photosynthetic pigments, and biochemical analysis of cow pea (Vigna ungiculata L.Walp) was studied. The different concentrations (0, 10, 25, 50, 100, 150, 200.) of zinc solutions were used to study in this experiment. The higher growth of germination percentage, seedling growth, dry weight, photosynthetic pigments such as chlorophyll "a", chlorophyll "b" total chlorophyll, and carotenoid contents, biochemical analysis such as sugar, starch, protein, and amino acids, contents of cow pea (Vigna ungiculata L) was observed in 10% concentration of zinc application. However increasing concentrations of Zinc reduce the growth of Cow pea.

KEYWORDS**:** *Zinc, Growth, Cowpea, Bio-Chemicals, Pigments*

INTRODUCTION

Environmental pollution creates a greater health hazard to human, animals and plants with local, regional and global implications (Irshad *et al*., 1997). Pollution has adverse effects on biotic and abiotic components of land, water and air. Water pollution may cause from municipal, agricultural or industrial wastes containing organic and inorganic chemical substances, dissolved and suspended solids (Moeller, 2004). Increasing population growth and consequential increase in food demand had required intensive year round food production. So, irrigation of arable land and crops from water bodies is very important for ensuring food satisfactory level. Increasingly, effluent admixed in 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 modernization.

Heavy metal contamination of water and soil is the main environmental problem in many developing countries. Heavy metals are basically classified by their toxicity, persistence and difficulty to be removed. Some of the heavy metals 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 serious problem for ecological, evolutionary and environmental reasons (Nagajyoti *et al.*, 2008). High concentrations of heavy 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. Zinc is one of the first micronutrients as well as heavy metal recognized as essential for plants that transported to plant root surface through diffusion (Maqsood *et al*., 2009).

Zinc is a micronutrient and in case of its severe deficiency reflects the symptoms may last throughout the entire crop season (Asad and Rafique, 2000). Zn deficient plant also appears to be stunted and yellowing (Torun *et al.*, 2001; Asad and Rafique, 2002). The growth and grain yield can be improved by addition of Zn fertilization (Maqsood *et al.*, 2009). Bora and Hazarika, (1997) reported the seed yield can be improved by addition of Zn fertilization. Chen and Aviad, (1990) and Moniruzzaman *et al*., (2008) found that application of Zinc improved soil organic matter and resulted in increasing mustard growth and yields. Kutuk *et al.*, (2000) also suggested that the application of lower level of Zn has become necessary for improved crop yields. Mandal and Sinha, (2004) recommended application of ZnSO4 at the rate of 20 kg ha-1 for oilseeds including mustard for better growth and yield.

Cowpea (*Vigna unguiculata* L.) is a protein rich pulse, fodder and green manure crop. Cowpea is one of the oldest pulse crops in Asian and African tropics. Being its rich protein and containing many other nutrients it is known as vegetable meat. Cowpea is mainly grown in Africa , about 90 per cent of the total world acreage is in Africa. It is also grown in Asia, North and South America, Australia, Central and Southern parts of Europe. As a legume, cowpea fixes substantial amounts of atmospheric nitrogen to meet its requirement. In India it is cultivated mainly in UP, M P, Bihar, Punjab, Haryana , Rajasthan , HP etc, where it is grown for both vegetable and pulse purposes and is a highly remunerative crop. Review of existing literature indicates that there is no consensus of opinion on the desirability of inorganic nitrogen for cowpea. Pulses contain a higher percentage of quality protein nearly three times as much as cereals, thus they are cheaper source to overcome protein malnutrition among human being.

MATERIALS AND METHODS

The Cowpea (*Vigna unguiculata* L.) seeds were obtained from Tamil Nadu Agricultural University, Vamban, Pudukkottai District, Tamilnadu. The seeds are uniform size, color was selected for the experimental purpose.

Zinc sulphate was used as the source of preparation of stock solution of Zinc. 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 were maintained as the control.

Shoot Length and Root Length

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.

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. K× B ×Total leaf area = L 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 C for 24 hrs. and then their dry weight were also determined over at 80 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.

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

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

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

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.

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

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 Folinphenol 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 C. The net°supernatant was saved. Then, the ethanol was evaporated in water both at 50

EXPERIMENTAL RESULTS AND DISCUSSIONS

Morphological Growth Parameters

The effect of different concentrations of zinc on seed germination and seedling growth of cow pea is given in Table 1. The higher germination percentage (100.0), root length (10.5 cm/seedling), shoot length (14.5 cm/seedling), and the dry weight (0.208 g/seedling) were recorded in 50 ppm zinc treatment. The lower germination percentage (56.0), root length (5.3 cm/seedling), shoot length (7.2 cm/seedling) and dry weight (0.104 g/seedling) were recorded in 200 ppm zinc treatment. The F values for the variance between the treatments were significant at 0.05 per cent level. The higher Vigour index (2450.0) was observed in 50 ppm. Similarly the higher tolerance index (0.9809) and percentage of phytotoxicity (49.52) were recorded in 10 ppm zinc and 200 ppm zinc respectively. The reduction in seedling growth might be due to the deleterious effects of heavy metals on the hydrolytic enzymes present in the storage organs as observed in other crops (Sheoran *et al.,* 1990; Geetha and De-Britto, 1998). The reduction of dry weight of cow pea seedlings under zinc stress was due to poor growth of seedlings in agreement with the results of Gensemer and Playle, 1999, Shikazono *et al.*, 2008, Sagardoy *et al.*, 2009, Cakmak 2010 and Barlog *et al.*,2016. A decrease in biomass productivity might be attributed to a disruption in nitrogen metabolism of seedlings under zinc stress (Chatterjee and Chatterjee, 2000; Ivanov *et al.,* 2011).

Table 1: Effect of Different Concentrations of Zinc on Germination Studies Of Tolerant Variety of Cowpea (*Vigna Unguiculata.* L)

Concentrations	Germination	Root Length	Shoot Length	Seedling Dry Weight	Vigour Index	Tolerane Index	Percentage of Phytotoxicity
Control	92 ±2.7	9.0 ±0.27	11.0 ±0.33	0.150 ±0.04	1711.2		
10	98 ±2.0	10.3 ±0.30	13.5 ±0.40	0.178 ±0.05	2263.8	0.8571	14.2857
25	96 ±2.8	9.6 ±0.28	12.0 ±0.36	0.159 ±0.04	2054.4	0.9104	1.9047
50	100 ±2.5	10.5 ±8.31	14.5 ±0.43	0.208 ±0.06	2450.0	0.9809	8.5714
100	88 ±2.6	8.2 ±0.24	10.8 ±0.32	0.132 ±0.03	1478.4	0.7809	21.9047
150	76 ±2.2	8.0 ±0.24	8.8 ±0.26	0.128 ±0.03	994.0	0.7619	23.8095
200	56 ±1.6	5.3 ±0.15	7.2 ±0.21	0.104 ±0.02	640.0	0.5047	49.5238
Standard deviation.							

Photosynthetic Pigments

The effect of different concentrations of zinc on the pigment contents of cow pea seedlings is presented in Fig. 1. The highest chlorophyll ‘a’, chlorophyll ‘b’ and total chlorophyll (0.295, 0.280 and 0.575 mg/g fr. wt.) and carotenoid (0.376 mg/g fr. wt.) contents were recorded in 50 ppm zinc treated seedlings. The lowest contents of chlorophyll ‘a’, chlorophyll ‘b’ and total chlorophyll (0.220, 0.195 and 0.415 mg/g fr. wt.) and carotenoid (0.158 mg/g fr. wt.) were recorded in 200 ppm zinc concentrations. The F value for the variance between the treatments was significant at 0.05 per cent level. Zinc stress is one of the important factors that affect photosynthesis in terms of CO_2 fixation, electron transport, phosphorylation and enzyme activities (Clijsters and van Assche, 1985; Shanker *et al.*, 2005).

The higher concentrations of zinc severely affected the carotenoid biosynthesis. Similar findings of the decline in carotenoid content were reported in various test crops (Corradi *et al.*, 1993; Nicholas *et al.*, 2000; Tripathi and Smith, 2000; Samantary and Deo, 2004; Rai *et al.*, 2004; Shanker *et al.*, 2005). Similar findings of Sugar content were reported in various crops such as *Phaseolus vulgaris* (Chaoui, *et al.*, 1997),*Bacopa monniera* (Ali *et al.*, 1999) and *Beta vulgaris* (Barłog *et al.*,2016). The decline in sugar formation may be associated with reduced rates of photochemical activities and chlorophyll formation. Loss of sugar formation may also be due to the conversion of sugar into energy when the plants were stressed (Outridge and Noller., 1991)

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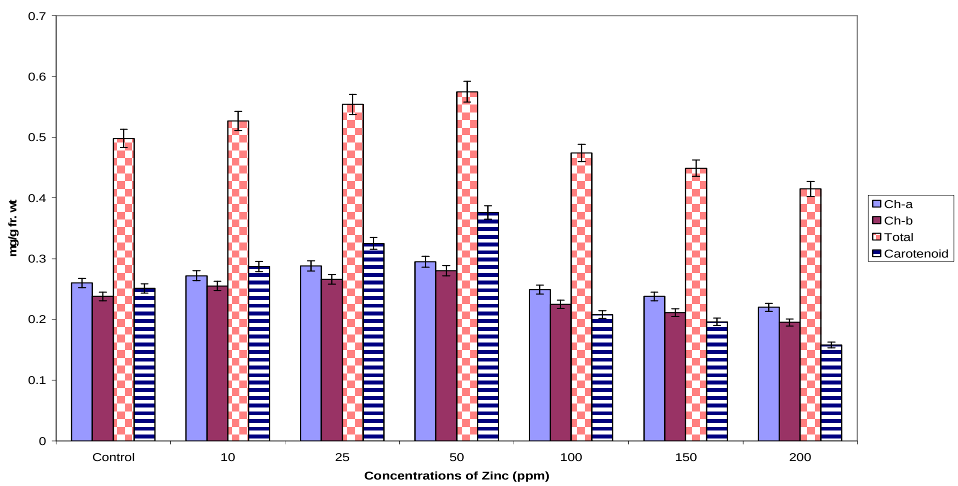

Figure 1: Effect of Different Concentrations of Zinc on Chlorophyll and Carotenoid Contents of Cow Pea.

BIOCHEMICAL ANALYSES

Sugars

The sugar content in root and shoot portion of cow pea are presented in Fig. 2. The higher amount of total sugar content (8.50 and 9.20 mg/g fr. wt.) was recorded in 50 ppm seedlings and lower amount (3.65 and 4.25 mg/g fr. wt.) was recorded in both root and shoot in 200 ppm zinc treated seedlings. The F values for the variance between the treatments were significant at 0.05 per cent level.

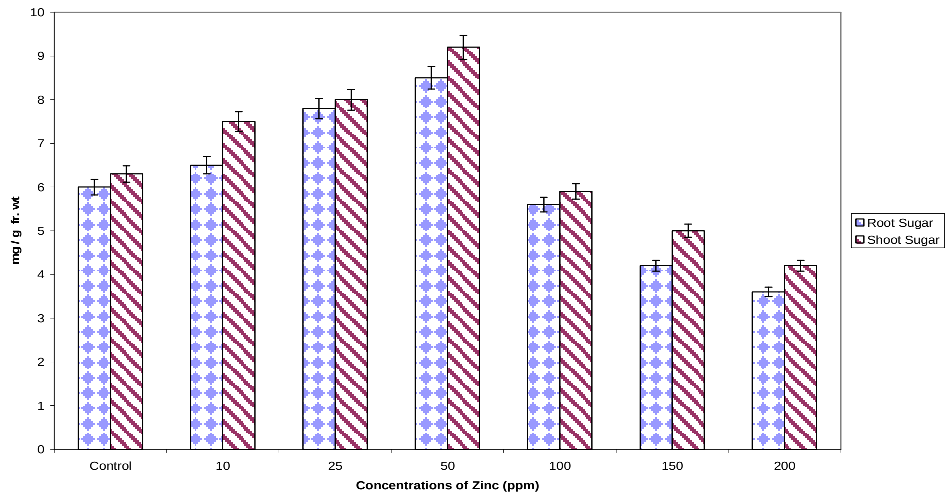

Figure 2: Effect of Different Concentrations of Zinc on Sugar Contents of Cow Pea.

Starch

The starch content of the cow pea root and shoot is presented in Fig. 3. The gradual decrease in starch content was recorded with increasing zinc concentrations. However 50 ppm zinc level produced positive effect on the sugar content. .The highest starch content in root (2.60 mg/g fr. wt.) and in shoot (3.81 mg/g fr. wt.) was recorded in control. At the same time, the lowest starch content of root (0.80 mg/g fr. wt.) and shoot (1.20 mg/g fr. wt.) were recorded at 200 ppm zinc treatment. The F values for the variance between the treatments were significant at 0.05 per cent level.

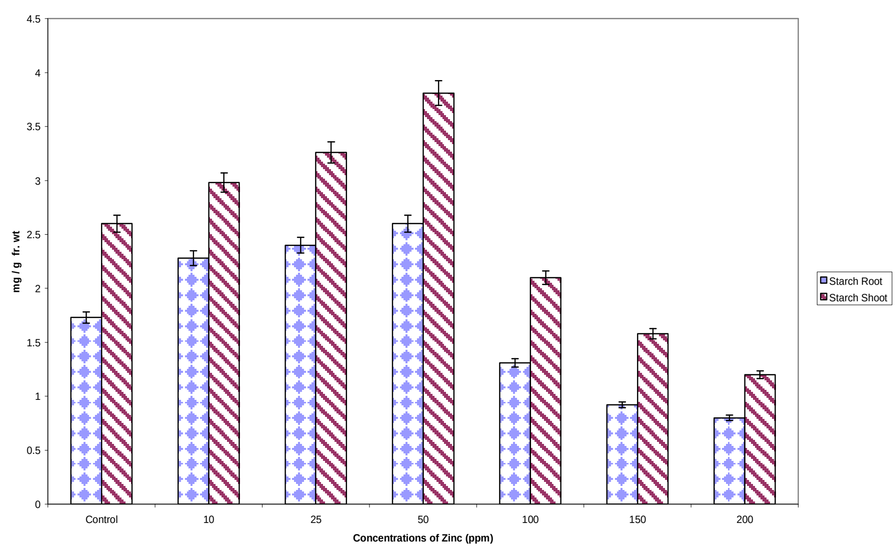

Figure 3: Effect of Different Concentrations of Zinc on Starch Content of Cow Pea.

Protein

The effect of different concentrations of zinc on the protein content of cow pea root and shoot is given in Fig. 4. The protein content of cowpea root was high (2.72 mg/g fr. wt.) in 50 ppm and low (1.13 mg/g fr. wt.) in 200 ppm zinc concentrations. Similarly, the highest and the lowest protein content of shoot (3.50 and 1.72 mg/g fr. wt.) were observed in 50 ppm and 200 ppm zinc concentrations. The F value for the variance between the treatments was significant at 0.05 per cent level. The effect of zinc on plant may decrease the total concentration of protein. (Chatterjee *et al*., 2004 and Mishra *et al*., 2006). This quantitative decrease in total protein content is the result of several zinc effects, acute oxidative stress of reactive Oxygen species (Piotrowska *et al.*, 2009),

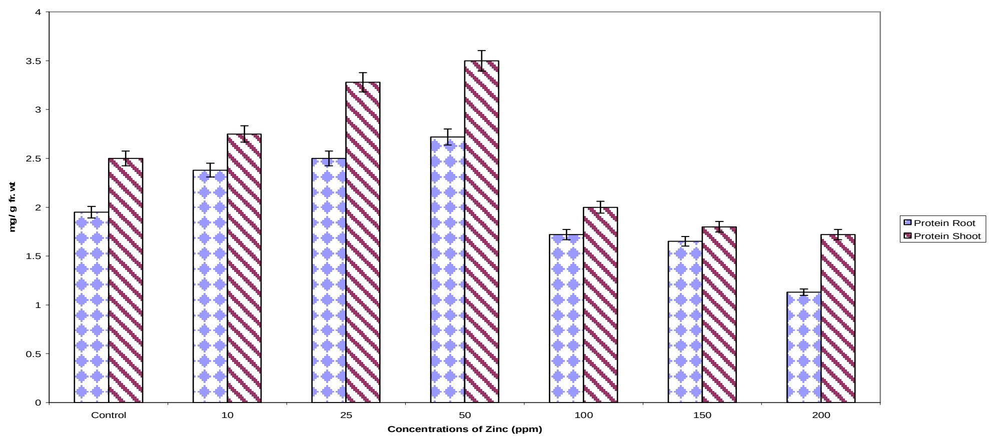

Figure 4: Effect of Different Concentrations of on Protein Content of Cow Pea.

Amino Acid

Amino acid content of root and shoot of cow pea seedlings is presented in Fig. 5. The highest amino acid content of root (3.60 mg/g fr. wt.) and shoot (4.28 mg/g fr. wt.) were recorded in 50 ppm. Similarly, the lowest amino acid content was recorded in the root (1.95 mg/g fr. wt.) and shoot (2.00 mg/g fr. wt.) at 200 ppm zinc treatment. The gradual decline of amino acid content was observed with increasing zinc concentrations. The F values for the variance between the treatments were significant at 0.05 per cent level. The effect of zinc on plant may decrease the total concentration of protein. (Chatterjee *et al*., 2004 and Mishra *et al*., 2006). This quantitative decrease in total protein content is the result of several zinc effects, acute oxidative stress of reactive Oxygen species (Piotrowska *et al.*, 2009),

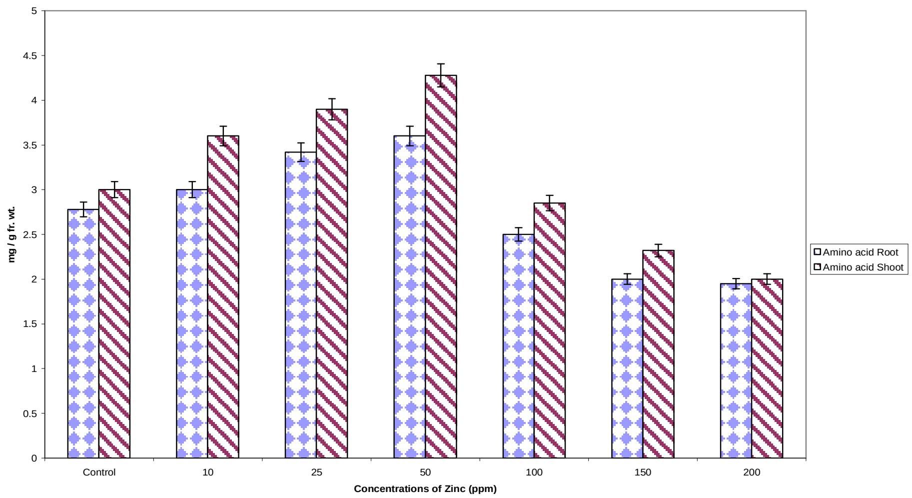

Figure 5: Effect of Different Concentrations of Zinc Onamino Acid Content of Cow Pea.

CONCLUSIONS

Zinc is a heavy metal. It is toxic to environment especially in plant community. The lower dose of zinc is favors for plant growths. The high level of zinc above 200 ppm is proved to be lethal to cow pea crop. However, zinc contaminated water can be properly treated and then discharged into nearby water bodies in order to prevent water pollution. Both government and public sector should join hands in the creation of a clean and green environment.

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