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Gosangi Avinash
Department of Biochemistry and Biochemical Engineering, Jacob School of Biotechnology and Bioengineering, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India

Shailendra K Srivasatava
Department of Biochemistry and Biochemical Engineering, Jacob School of Biotechnology and Bioengineering, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India

Divya Singh
Department of Biochemistry and Biochemical Engineering, Jacob School of Biotechnology and Bioengineering, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India

Correspondence
Gosangi Avinash
Department of Biochemistry and Biochemical Engineering, Jacob School of Biotechnology and Bioengineering, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, India

Effect of copper on various phytochemical in *Sorghum bicolor*

Gosangi Avinash, Shailendra K Srivasatava and Divya Singh

Abstract
Copper (Cu) is one of the micronutrients needed by plants which activates some enzymes in plants, also required in the process of photosynthesis is essential in plant respiration and assists in metabolism of carbohydrates and proteins, however excess of copper inhibits plant growth and impairs important cellular process (i.e. photosynthesis electron transport). In recent years cu pollution in agricultural soils due to arbitrary use of pesticides, fungicides, industrial effluent. Here we reviewed the adverse effects of cu on Sorghum bicolour was investigated. Copper was applied in the form of copper sulphate ($CuSo_4.5H_20$) in four levels (0, 5.0, 10.0 and 15.0ppm. we observed visible symptoms of cu toxicity in this plants. During the exposure of plants to excess copper the antioxidant defense system the plant to protect itself from the damage up to some extent with increasing copper concentration of Ascorbic Acid activity increased in leaves compared with that of control group up to 10.0ppm concentration of Cu. Total phenol content in the leaves showed reduction with increasing cu concentration as compared to control at days. The chlorophyll amount gradually declined with Cu concentrations. There was gradual decrease in the content of phytochemicals. (Tannin, Saponin, Alkaloid).

Keywords: Copper, chlorophyll, cardiac glycosides, TPC flavonoids Ascorbic Acid, Tannin, tocopherol, *Sorghum bicolor*

1. Introduction
Copper is an essential metal for normal plant growth and development having Atomic number, Density and Weight respectively 29, 8.92 g/cm^3 and 63.55. It represented by "Cu" in periodic table. At room temperature is in crystalline form but melts at 1,084.62 °C and boils on 5,301 °F. Copper is having a large number of isotopes *i.e.* 35 in which 2 are stable. Most common isotopes: Cu-63 (69.15 percent natural abundance) and Cu-65 (30.85 percent natural abundance) Copper is a reddish metal that occurs naturally in rock, soil, water, sediment, and, at low levels, air. Its average concentration in the earth's crust is about 50 parts copper per million parts soil (ppm) or, stated another way, 50 grams of copper per 1,000,000 grams of soil (1.8 ounces or 0.11 pounds of copper per 2,200 pounds of soil). Copper also occurs naturally in all plants and animals. It is an essential element for all known living organisms including humans and other animals at low levels of intake. Copper ranks as third-most-consumed industrial metal in the world, after iron and aluminium, according to the U.S. Geological Survey. Copper concentrations in soil generally contains between 2 and 250 ppm and healthy plant tissues range from 20 to 30 μg g^{-1} dry weight (dw) (Hodenberg and Fink 1975). In particular, free copper ions can catalyze the formation of highly toxic reactive oxygen species (ROS) such as hydroxyl radicals ($OH^{\bullet}$) from superoxide anions ($O_2^{\bullet-}$) or hydrogen peroxide (H_2O_2) via the Haber–Weiss reaction. However, in excess, copper can interfere with numerous physiological processes such as enzyme activity, DNA alterations, proteins oxidation, and membrane integrity, all of which could lead to growth inhibition of plant (Alaoui *et al.*, 2004) [3]. Plant concentrations of essential elements may exceed the critical the minimum concentrations required for growth and may vary somewhat from species to species. Nonetheless, the following value gives the general requirement of plants. Typical concentrations sufficient for plant growth. Copper 6mg/kg relative number of atoms 100.Toxicity to humans: 700-2100 mg/g dry liver tissue = lethal. The permissible limit of copper for plants is 10mg/kg recommended by WHO (Zigham *et al.*, 2012). In all the collected plant samples concentration of copper was recorded below the permissible limit. The maximum permissible limit for Cu in water is 2 mg/l in water samples concentration of copper ranged between 0.258 to 0.659mg/l. In all the collected water samples concentration of copper was recorded below the permissible

limit. Contamination of drinking water with high level of copper may lead to chronic anaemia**.** Copper accumulates in liver and brain. Copper toxicity is a fundamental cause of Wilson's disease. Copper particulates are released into the atmosphere by windblown dust; volcanic eruptions; and anthropogenic sources, primarily copper smelters and ore processing facilities. The fate of elemental copper in water is complex and influenced by pH, dissolved oxygen and the presence of oxidizing agents and chelating compounds or ions. Concentration of copper in all the soil samples was above the maximum permissible limit set by WHO. Concentration of copper ranged between 0.536-1.504mg/kg. Copper is an essential trace element in plants and animals, but not some microorganisms. The human body contains copper at a level of about 1.4 to 2.1 mg per kg of body mass (Adelstein *et al.*, 1961) [1, 2]

Toxic levels of Cu occur naturally in some soils whereas others may contain high levels of Cu as a result of the anthropogenic release of heavy metals into the environment through mining, smelting, manufacturing, and agriculture waste disposal technologies. At concentrations above those required for optimal growth Cu was shown to inhibit growth and to interfere with important cellular processes such as photosynthesis and respiration (Prasad and Strzalka, 1999) [17]. Hence, the presence of excess Cu can cause oxidative stress in plants and subsequently increase the antioxidant responses due to increased production of highly toxic oxygen free radicals. Accordingly, it was observed that excess Cu in plants led to oxidative stress inducing changes in the activity and content of some components of the antioxidative pathways (*i.e.*, ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), superoxide dismutases (SODs), guiacol peroxidase) (De Vos *et al.*, 1992) [8] Considering that Cu is an efficient catalyst in the formation of reactive oxygen species (ROS), it was suggested that the increased Cu toxicity by light during photo inhibition is due to production of hydroxyl radicals (Yruela *et al.*, 1996) [25, 26]. Phenolic compounds in sorghum occur as phenolic acids, flavonoids and condensed tannins. The antioxidative phytochemicals in grains, vegetables and fruits have received increased attention recently for their potential role in prevention of human diseases as well as in food quality improvement.

2. Materials and methods

2.1 Treatments of plants

In the present study *Sorghum bicolor* plants used to assess the effect of copper in three different concentrations *i.e.* 5ppm, 10ppm and 15ppm along with control.

2.2 Collection and Preparation of Samples

Leaf samples were collected for every 15 days interval up to three harvests respectively from all the groups i.e. control, 5ppm, 10ppm, 15ppm, copper treated plants.

2.3 Sample preparation

5g of fresh leaf sample/1 g of dried leaf sample was homogenised with 25 ml of 0.1 M phosphate buffer pH 7.0 and/or corresponding solvents in ice-cold sterile pestle and mortar at 4 °C. The homogenate was centrifuged at 10000rpm for 20 minutes. Supernatant was collected as crude enzyme.

2.4 Estimation of chlorophyll a, b, total chlorophyll content

Treated and untreated leaves (100mg) were crushed in 5ml of (80%v/v) chilled acetone by the method of Arnon (1949) [4]. Extract was centrifuged at 10000 rpm for 10 min and absorbance of the supernatant was read at 510nm, 663nm, 665nm and using *uv-vis* spectrophotometer.

Calculation

$$\text{Chlorophyll a (mg g-1 fw)} = \frac{\{12.7(A663)-2.63(A645)\}V}{1000\times W\ (g)}$$

$$\text{Chlorophyll b (mg g-1 fw)} = \frac{\{22.9(A645)-4.68(A663)\}\ V}{1000\times W\ (g)}$$

Total chlorophyll (mg g-1fw)

$$= [20.2(A_{645}) + 8.02(A_{663})]\ v/1000\times W_{(g)}$$

Where: A_{510} and A_{480}=absorption at these wavelengths
V=Final extract volume (ml)
W=Weight of sample (g)

2.5 DPPH radical – scavenging activity

Scavenging'' radical capacity of samples was determined using DPPH redical (Hatano *et al.*, 2001). Reaction mixtures (0.001 to 1 mg/ml) of samples were prepared by mixing appropriate amounts of extract, 2.5ml of DPPH and methanol to a total volume of 10ml. Prepared solutions were left in the dark for 60 minutes and then the absorbance was measured at 515 nm. All determinations were performed in triplicate. Methanol was used to zero spectrophotometer methanolic solutions pure compounds (gallic acid, BHT, Trolox, ascorbic acid, rutin and quercethin) were tested too at different concentration. DPPH radicals" scavenging "capacity was expressed by applying the following equation DPPH – RSC (%) = 100 × {A0-A1} Ao- absorbance of blank solution, A1-absorbance of solution in the present of active components.

2.6 Standard vitamin C (Ascorbic acid) solution

Solution of 500 ppm of vitamin C was prepared fresh.

2.7 Sample preparation

Five grams of sample were homogenized with 25 ml of metaphosphoric acid - acetic acid solution, and it was quantitatively transferred into a 50 ml volumetric flask and shaken gently to homogenize solution. Then it was dilute up to the mark by the metaphosphoric acid - acetic acid solution. The obtained solution is filtered and centrifuged at 4000 rpm for 15 minutes, after that the supernatant solution is used for spectrophotometric determination.

2.8 Procedure

Bromine water (0.23 ml of 3%) were added into 4 ml of centrifuged sample solution to oxidize the ascorbic acid to dehydroascorbic acid and after that 0.13 ml of 10% thiourea to remove the excess of bromine. Then 1 ml of 2, 4- DNPH solution was added to form osazone. All standards, samples and blank solution were kept at 37 °C temperature for 3 hours in a thermostatic bath. After it all were cooled in ice bath for 30 minutes and treated with 5 ml chilled 85% H_2SO_4, with constant stirring. As a result, a coloured solution's absorbance was taken at 521 nm. Calculation is Amount of vitamin C in the sample was determined from the standard curve and the result was expressed as mg/100 gm.

2.9 Estimation of vitamin E (Rutkowski *et al.*, 2005) [19]

Measure 0.5 ml of the analysed fluid into the test-tube I

(centrifugal) with a tight stopper, add 0.5 ml of anhydrous ethanol and shake vigorously the plugged test tube for 1 minute add 3 ml xylene, plug the test tube and shake vigorously for another 1 minute centrifuge the tube to separate the extract (1500×g 10 minutes); simultaneously measure 0.25 ml solution of phenanthroline into a usual test-tube II**.** collected 1.5 ml of the extract (upper layer), transfer to the test-tube II and mix the content Added 0.25 ml of FeCl3solution to the test tube II, mix, add 0.25 ml of H3PO4 solu-tion and mix again – this way a test sample is obtained for spectrophotometric measurements. Prepare the standard sample (0.5 ml of the standard solution instead of the analysed liquid): using Trolox – prepare as the test sample, using α-tocopherol – add 0.5 ml of DI water instead of anhydrous ethanol at the beginning of the analysis; do not centrifuge this sample. Measure absorbance of the test sample Axand of the standard sample Asat 539 nm against the blank test (preparation – as the test sample but using water instead of the analysed liquid). Calculate concentration cx of vitamin E (μM) in the analysed liquid, using the a/a presented formula.

2.10 Estimation of Phytochemical

2.10.1 Estimation of total phenolic content

Total phenol content in the samples was estimated by the method of Hossain *et al*., (2013 Reagents: 80% methanol, Folin-Ciocalteu reagent (FCR), 20% Na_2CO_3, Standard (100 mg catechol in 100 ml of water).

2.10.2 Procedure

Tissue (1g) was finely chopped into small pieces and refluxed in 80% methanol for 10 min. After cooling, the tissue was homogenized with mortar and pestle. The homogenate was filtered and centrifuged at 10000 rpm for 10 min. The supernatant was used for the estimation of total phenols. An aliquot of the sample was pipetted out and made up to 3 ml with 80% methanol. Folin-ciocalteau reagent (0.5 ml) was added and kept for 3 min. 2 ml, 20% $Na_2 CO_3$ was added to the mixture and kept in boiling water bath for 1 min. The white precipitate was removed by centrifuging for 10 min and the absorbance of the clear light blue solution was recorded at 650 nm against the reagent blank containing 3 ml. 80% methanol, 0.5 ml Folin's reagent and 2 ml 20% $Na_2 CO_3$ The reaction between phenols and an oxidizing agent phosphomolybdate in Folin-Ciocalteau reagent resulted in the formation of a blue complex. A standard graph of phenols was constructed with pyrocatechol by taking absorbance against different concentration. Calculation of total phenols g-1 tissue was calculated from the standard.

2.11 Estimation of Tannin (Swain 1959) [22, 23]

2.11.1 Preparation of standard tannic acid solution

Tannic acid (100 g) was dissolved in 100 ml distilled water. Preparation of working solution 5 ml stock solution was diluted to 100 ml with distilled water. Each ml contained 50μg of tannic acid.

2.11.2 Method Preparation of standard curve

10ml of standard solution was made up to 100ml distilled water. 1 -10ml aliquots were taken in clear test tubes. 0.5ml of Folin-Denis reagent and one ml of sodium carbonate solution was added to each tube. Each tune was made upto 10 ml with distilled water. All the reagents in each tube were mixed well and kept undisturbed for about 30 minutes and read at 760 nm against reagent blank.

2.11.3 Extraction of Tannin

Accurately Weighed 0.5g of the powdered material was transferred to a 250mL conical flask. Add 75mL water. Heat the flask gently and boil for 30 min. Centrifuged at 2,000rpm for 20 min and collect the supernatant in 100mL volumetric flask and make up the volume. Transfer 1mL of the sample extract to a 100mL volumetric flask containing 75mL water. Add 5mL of Folin-Denis reagent, 10mL of sodium carbonate solution and dilute to 100mL with water and Shaken well. Read the absorbance at 700nm after 30 min. Estimation of sample an aliquot of the sample extract containing not more than 0.5mg of tannic acid was used and the percentage of tannin was determined.

2.12 Determination of Cardiac glycosides

El-Olemy *et al.*, 1994 Buljet's reagents was used to evaluate the cardic glycoside content in the examined plant parts. For this purpose, 1 g of each powdered sample was soaked in 100ml of 70% alcohol for 2 hrs before filtration. Using lead acetate and Na2HPO4 solution, the obtained extracts were purified before the addition of freshly prepared Buljet's reagent. The difference between the intensity of colours of the experimental and blank samples (distilled water and buljet's reagent) gave the absorbance, which is proportional to the concentration of glycosides.

2.13 Estimation of flavonoid (Swian 1959) [22, 25]

The plan extracts (50mg) were dissolved separately in 50 ml of methanol these solutions were serially diluted with methanol to obtain lower dilutions phloroglucinol 50(mg) was dissolved in 50ml of distilled water. It was serially diluted with water to obtain lower dilutions. 0.2ml of the extract was taken in a test tube and the final volume was made up to 2 ml with distil water To this 4ml of vanillin reagent was added rapidly. Exactly after 15mn absorbance was recorded at 500nm against blank the unknown was read from a standard curve prepared using different concentration of phosphoglycerol.

2.14 Statistical analysis

The data recorded during the course of investigation were subjected to statistical analysis by "Analysis of variance" technique (Fisher and Yates, 1963) for drawing conclusions. The significant and non-significant treatment affect was tested against the critical difference at 5% level. The significant difference between the means was tested against the critical difference at 5% level. For testing the hypothesis, the following ANOVA table was used (Chandel 2002).

3. Result and discussion

3.1 Effect of the Copper on Chlorophyll a, b, and total Chlorophyll content in *Sorghum bicolor* (L.) leaves

The concentration scrutiny of the data demonstrated decrease in chlorophyll a, b, and total chlorophyll content in the leaves, with increasing concentration of Cu upto 15.0 ppm after 15days from sowing as compared to control. The maximum decrease of 55.68%, 64.12% and 70.06% was observed at 15ppm concentration of Cu after the exposure period respectively in first harvest. However, during days of exposure period, the chlorophyll a, b and total chlorophyll content was found to decrease with increase in concentration of Cu. Maximum decrease of 51.36%, 52.29% and 55.71% was observed at 15.0 ppm after 30days (2^{nd} harvest) of exposure as compared to respective control. A decrease in chlorophyll content was found to be significant on increasing

copper concentration. This observation was also supported by Chandra and Alam, (2013) [18] in the study copper scavenging potential and its effect on chlorophyll in seedlings of *Brassica juncea* (L.) The decrease in chlorophyll content may be due to reduced chlorophyll biosynthesis by inhibiting δ-amino levulinic dehydrogenase and protochlorophyllide reductase activities and breakdown of pigments or their precursor as reported by Teramura and Sullivan in 1994.

3.2 Total phenol content

The concentration wise analysis of the result of total phenolin the leaves showed reduction with increasing cu concentration as compared to control at days. The maximum decrease of 59.4%, and 59.3% for 1st, and 2nd harvest respectively at 15ppm concentration of cu was observed at days as compared to control as shown in Figure no. 4.5 and table no. 4.5. According to Dayangku *et al.*, (2015) [7]. Total phenolic content in the callus treated with 200 µM copper, compared to control. Although there was a slight reduction in total phenolics content when cells were exposed to 250 µM copper, in the experiment Effects of Copper on Total Phenolics, Flavonoids and Mitochondrial Properties of *Orthosiphon stamineus* Callus Culture. Phenolic compound can be involved in the H_2O_2 scavenging cascade in the plants cells. (Takahama and Oniki, 1997). Phenolics have various functions in plants. An enhancement of phenylopropanoid metabolism and the amount of phenolic compounds can be observed under different environmental factors and stress conditions. The induction of phenolic compound biosynthesis was observed in wheat in response to nickel toxicity (Diaz *et al.*, 2001).

3.3 Cardiac glycosides

The concentration wise analysis of the data of Cardiac glycosides in the leaves showed reduction with increasing cu concentration as compared to control. The maximum decrease of 51.36%, 1st harvest) and (69.3%, at 3rd harvest) in 15ppm Cu treated plants as shown in figure no. 4.6& 4.7 and table no. 4.6&4.7.

3.4 Saponin

Saponin content was observed to be decreased in case of 15.0ppm Cu treated plants (52.29%, 1st harvest) and (50.46% at 3nd harvest) as compared to control as shown in figure no. 4.6 & 4.7 and table no. 4.6 &4.7. The decrease in saponin, antioxidant enzymes and biomass at higher concentration of Cu may be ascertained to the toxic effect of Cu to growth as well as saponin accumulation. Awate and Gaikwad (2014) reported that saponin content increased in stressed plants as compared to control plants. Therefore, enhancement of saponin and antioxidant enzyme at low level of Cu suggesting that they provides an intrinsic defense to resist Cu-induced oxidative damage in *P. ginseng* plant. Mohammod *et al.*, (2006).

3.5 Tannin

The concentration wise analysis of the result oftanninin the leaves showed reduction with increasing cu concentration as compared to control. The maximum decrease of 47.80%, a 52.87% and 38.69% for 1st, 2nd, and 3rd harvest respectively at 15ppm concentration of cu was observed at days as compared to control as shown in figure no 5.0and table no 5.0.

3.6 Amount of Flavonoid

The concentration wise analysis of the Amount of Flavonoid in the leaves showed reduction with increasing cu concentration as compared to control at days. The maximum decrease of 65.27%, and 65.35% for 1st, 3nd and harvest respectively at 15ppm concentration of cu was observed at days as compared to control as shown in figure no 5.1and table no 5.1. According to Andresa *et al.*, (2014), increase in concentration of Cu in the environment led to a reduction in weight, significant ($p< 0.05$) loss in water content, chlorophyll, carotenoids, phenolic compounds, proline, anthocyanins, flavanoids, and soluble sugars. In the experiment Short-term physiological responses to copper stress in *Salvinia auriculata.*

3.7. Activity of Ascorbic Acid

The concentration wise analysis of the result of ascorbic acid activity in the leaves showed maximum increase of 95.75% and 82% for 1st,and IIInd harvest respectively at 10ppm and 79.94% for 2nd harvest at 15ppm concentration of cu was observed at days as compared to control as shown in figure no 5.7 and table no 5.7 According to Fikriye *et al.* (2005) [10] there was a significant increase in retinol, α- pherol and ascorbic acid Cd-treated seedlings stated in the experiment effects of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in bean (*phaseolus vulgarisl.*) seedlings.Ascorbic acid (AsA) is a key antioxidant, and involved in protection of plant cells against oxidative damage catalyzed by ROS. It acts as a chain breaking antioxidant impairs with the formation of free radicals in the process of formation of intracellular substances throughout the plants body (Prakash *et al*., 2009) [10]. It is essentiallyrequiredini) scavenging of H_2O_2. by acorbates – glutathione cycle, ii) elimination of ROS, iii) used maintenance of α-tocopherol in reduced form, and iv) utilized as a cofactor in xanthophylls cycle to protect chloroplast against photooxidative damage (Smirnoff, 2000) [21]

3.8. Activity of α-tocopherol

The concentration wise analysis of the result of α- tocopherol activity in the leaves showed maximum increase of 73.69%, 81.34% and 78.29% for 1st, 2nd and 3nd harvest respectively at 15.0 ppm concentration of cu was observed at days as compared to control as shown in figure no 5.8and table no 5.8. Alpha-tocopherol is the most active form of vitamin E and is synthesized in the plastids of higher plants. It is found to be involved in scavenging ROS and lipid peroxides (Munne *et al.*, 2005) [15] by quenching $^1O_2$ in the chloroplast and thus, prevents cell membrane from damage under stress. Several studies have reported changes in the levels of α-tocopherol under heavy metal stress Lushchak *et al.*, (2012) [14]. A study by Collin *et al.* (2008) [6] reported an increased concentration of α-tocopherol in *Arabidopsis* under Cd treatment, and the authors suggested that there is an upregulation of genes related to its biosynthesist Singh *et al.*, (2016) The alpha –tocopherol levels have been shown to increase under various stressful condition.

Table 1: Effect of Cu on the chlorophyll content (m mol $min^{-1}g^{-1}fw$) in *Sorghum bicolor* leaves (on 15th days from day of germination)

Treatments	Chlorophyll a	Chlorophyll b	Total chlorophyll
T_0 Control	0.334±0.013	0.407±0.009	0.481±0.019
$T_1$5.0 ppm	0.242±0.006	0.240±0.006	0.238±0.005
T_2 10.0 ppm	0.215±0.006	0.213±0.006	0.211±0.006
T_3 15.0 ppm	0.148±0.016	0.146±0.016	0.144±0.016
F- test	S	S	S
S. Ed. (±)	0.009	0.009	0.009
C.D.(P=0.05)	0.019	0.019	0.019

Table 2: Effect of Cu on the chlorophyll content (m mol $min^{-1}g^{-1}fw$) in *Sorghum bicolor* leaves (on 15th days from first harvest)

Treatments	Chlorophyll a	Chlorophyll b	Total chlorophyll
T_0 Control	0.220±0.020	0.218±0.018	0.210±0.026
$T_1$5.0 ppm	0.190±0.007	0.179±0.018	0.169±0.007
T_2 10.0 ppm	0.169±0.007	0.159±0.004	0.149±0.004
T_3 15.0 ppm	0.107±0.007	0.104±0.012	0.093± 0.005
F- test	S	S	S
S. Ed. (±)	0.008	0.008	0.010
C.D.(P=0.05)	0.017	0.018	0.021

Table 3: The effects of cu on the total phenol content (µ mol min^{-1} $g^{-1}fw$) in *Sorghum bicolor*. (15th and 45thDAS)

Treatments	1ST harvest	11nd harvest
T_0 Control	0.367+0.040	0.354±0.045
$T_1$5.0 ppm	0.280+0.046	0.274±0.044
T_2 10.0 ppm	0.215+1.042	0.217±0.010
T_3 15.0 ppm	0.149+0.	0.144±0.013
F- test	S	S
S. Ed. (±)	0.378	0.023
C.D.(P=0.05)	0.801	0.049

Table 4: The effects of Cu on the cardiac glycosides, saponin, and alkaloids content (µ mol min^{-1} $g^{-1}fw$) in *Sorghum bicolor*. (15th DAS)

Treatments	Cardiac Glycosides	Saponin	Alkaloid
T_0 Control	1.870±0.050	2.150±0.070	1.090±0.030
$T_1$5.0 ppm	0.899±0.040	1.630±0.050	1.060±0.030
T_2 10.0 ppm	0.810±0.150	1.580±0.140	1.130±0.040
T_3 15.0 ppm	0.563±0.020	1.065±0.120	0.803±0.020
F- test	S	S	
S. Ed. (±)	0.041	0.030	0.006
C.D.(P=0.05)	0.087	0.063	0.012

Table 5: The effect of Cu on the cardiac glycosides, saponin and alkaloid content (µ mol min^{-1} $g^{-1}fw$) in *Sorghum bicolor* (45th DAS)

Treatments	Cardiac Glycosides	Saponin	Alkaloid
T_0 Control	0.220±0.020	0.218±0.018	0.210±0.026
$T_1$5.0 ppm	0.190±0.007	0.179±0.007	0.169±0.007
T_2 10.0 ppm	0.169±0.007	0.159±0.004	0.149±0.004
T_3 15.0 ppm	0.107±0.007	0.104±0.012	0.093±0.005
F- test	S	S	
S. Ed. (±)	0.008	0.008	0.010
C.D.(P=0.05)	0.017	0.018	0.021

Table 6: The effect of Cu on the Tannin content (µ mol min^{-1} $g^{-1}fw$) in *Sorghum bicolor* (15th, 30th and 45th DAS)

Treatments	1st harvest	2nd harvest	3rd harvest
T_0 Control	0.182±0.010	0.191±0.010	0.199±0.010
$T_1$5.0 ppm	0.160±0.003	0.158±0.004	0.148±0.004
T_2 10.0 ppm	0.144±0.003	0.140±0.005	0.130±0.005
T_3 15.0 ppm	0.095±0.009	0.090±0.004	0.122±0.021
F- test	S	S	
S. Ed. (±)	0.005	0.005	0.010
C.D.(P=0.05)	0.011	0.010	0.021

Table 7: The effect of Cu on the Flavonoids content (µ mol min^{-1} $g^{-1}fw$) in *Sorghum bicolor leaves* (15th and 45th DAS).

Treatments	Ist Harvest	3rd Harvest
T_0 Control	10.410±0.055	9.400±0.071
$T_1$5.0 ppm	6.690±0.046	5.680±0.150
T_2 10.0 ppm	5.580±0.157	4.570±0.143
T_3 15.0 ppm	3.615±0.024	2.858±0.125
F- test	S	S
S. Ed. (±)	0.042	0.025
C.D.(P=0.05)	0.089	0.054

Table 8: The effects of Cu on the ascorbic acid content (µ mol min^{-1} $g^{-1}fw$) in *Sorghum bicolor*. (On 15th, 30th and 45th days from day of germination)

Treatments	Ist Harvest	2nd Harvest	3rd Harvest
T_0 Control	2.000±0.059	1.820±0.079	2.000±0.036
$T_1$5.0 ppm	2.730±0.049	3.185±0.450	3.190±0.035
T_2 10.0 ppm	3.915±0.159	2.355±0.540	3.640±0.046
T_3 15.0 ppm	2.526±0.029	3.275±0.520	3.270±0.026
F- test	S	S	S
S. Ed. (±)	0.041	0.152	0.006
C.D.(P=0.05)	0.087	0.323	0.012

Table 9: The effects of cu on theα- tocopherol content (µ mol min^{-1} $g^{-1}fw$) in *Sorghum bicolor* leaves (on 15th, 30th and 45th days from day of germination)

Treatments	Ist Harvest	2nd Harvest	3rd Harvest
T_0 Control	4.650±0.058	5.430±0.075	5.271±0.039
$T_1$5.0 ppm	3.580±0.048	3.315±0.054	3.245±0.038
T_2 10.0 ppm	2.140±0.158	2.140±0.149	2.335±0.048
T_3 15.0 ppm	1.223±0.028	1.013±0.149	1.144±0.028
F- test	S	S	S
S. Ed. (±)	0.041	0.031	0.006
C.D.(P=0.05)	0.087	0.066	0.012

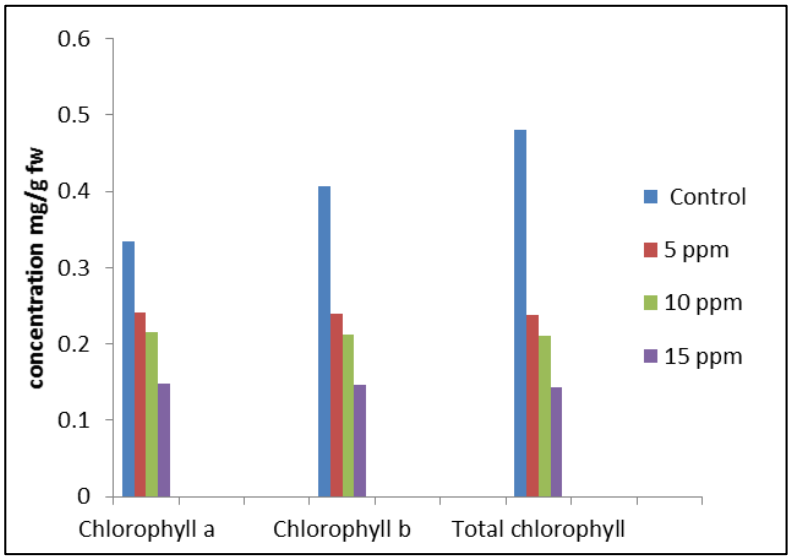

Fig 1: Effect of Cu on Chlorophyll content in *Sorghum bicolor* Leaves (1st harvest; 15th days from day of germination)

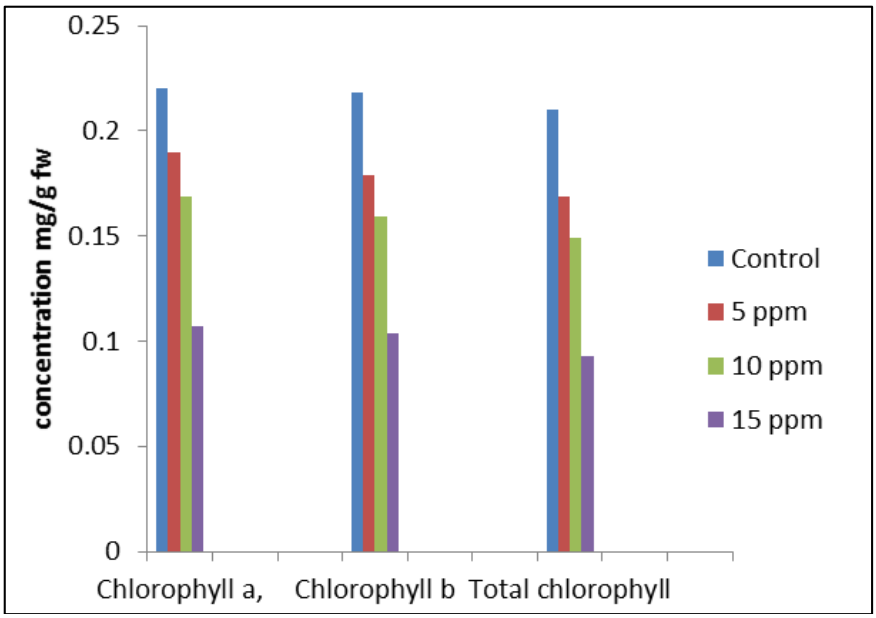

Fig 2: Effect of Cu on Chlorophyll content in *Sorghum bicolor* Leaves (2nd harvest; on 15th days from first harvest)

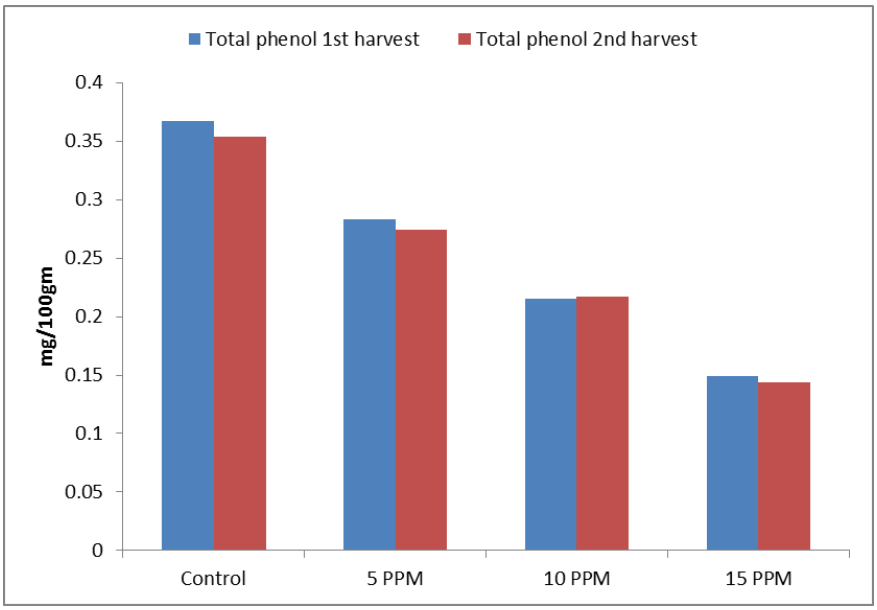

Fig 3: The effect of Cu on the total phenol content (µ mol min^{-1} g^{-1}fw) in *Sorghum bicolor leaves* (15th and 45th DAS)

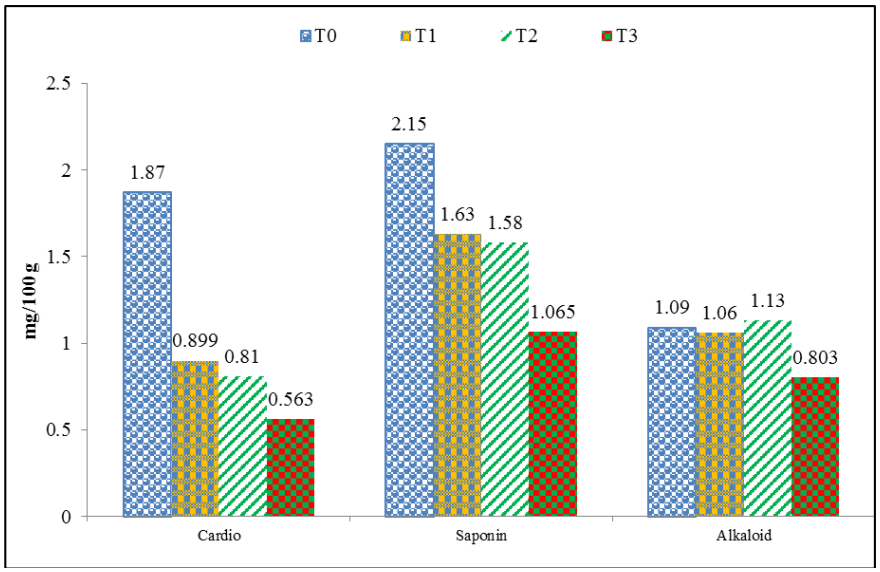

Fig 4: The effect of Cu on the cardiac glycosides, saponin and alkaloid content (µ mol min^{-1} g^{-1}fw) in *Sorghum bicolor* (15th days from day of germination)

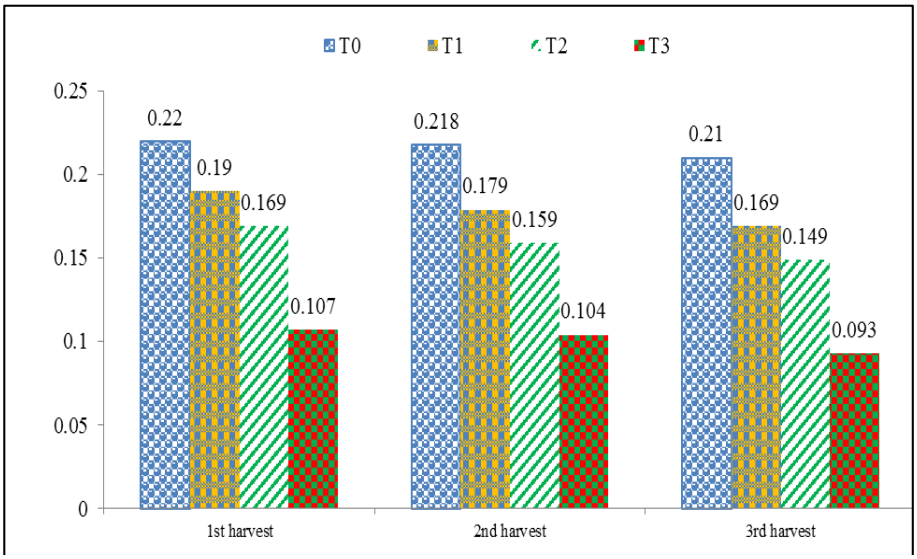

Fig 5: The effect of Cu on the cardiac glycosides, saponin and alkaloid content (mol min^{-1} g^{-1}fw) in *Sorghum bicolor leaves* (45^{th} DAS)

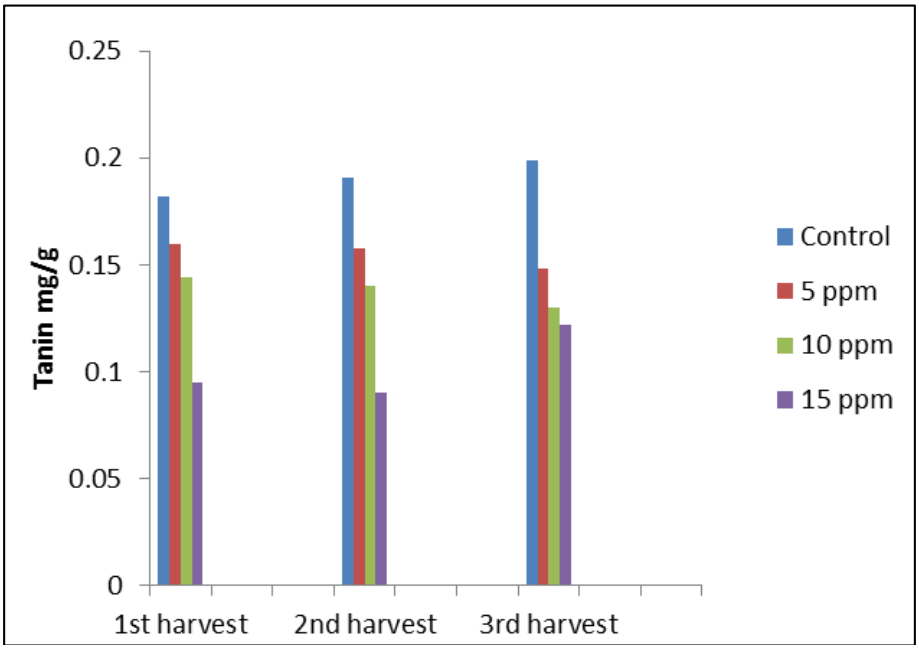

Fig 6: The effect of Cu on the Tannin content (µ mol min^{-1} g^{-1}fw) in *Sorghum bicolor* (15th, 30th and 45^{th} days from day of germination)

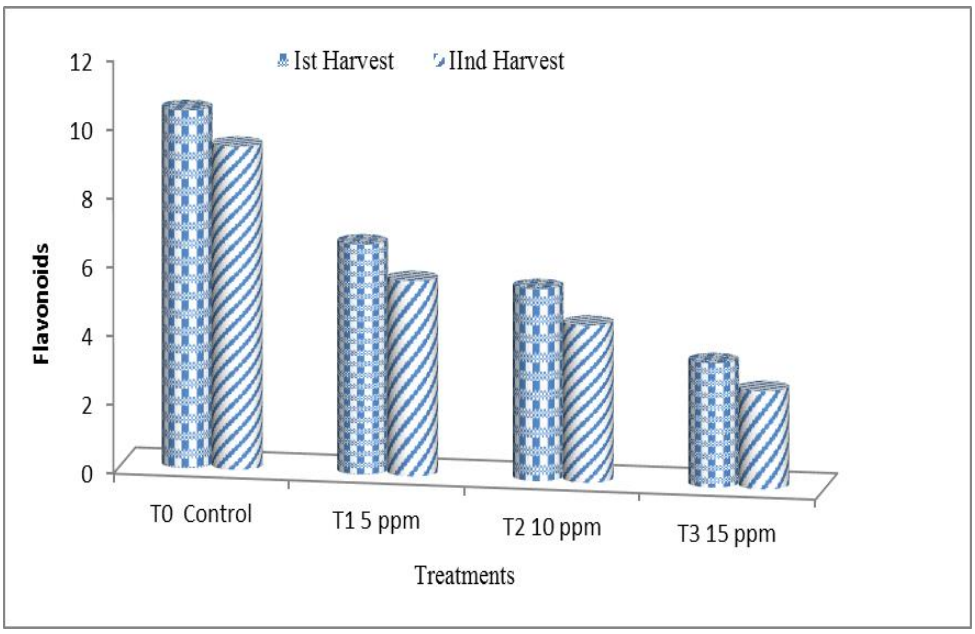

Fig 7: The effect of Cu on the Flavonoids content (µ mol min^{-1} g^{-1}fw) in *Sorghum bicolor* ($15^{th,}$ 30^{th} and 45^{th} DAS)

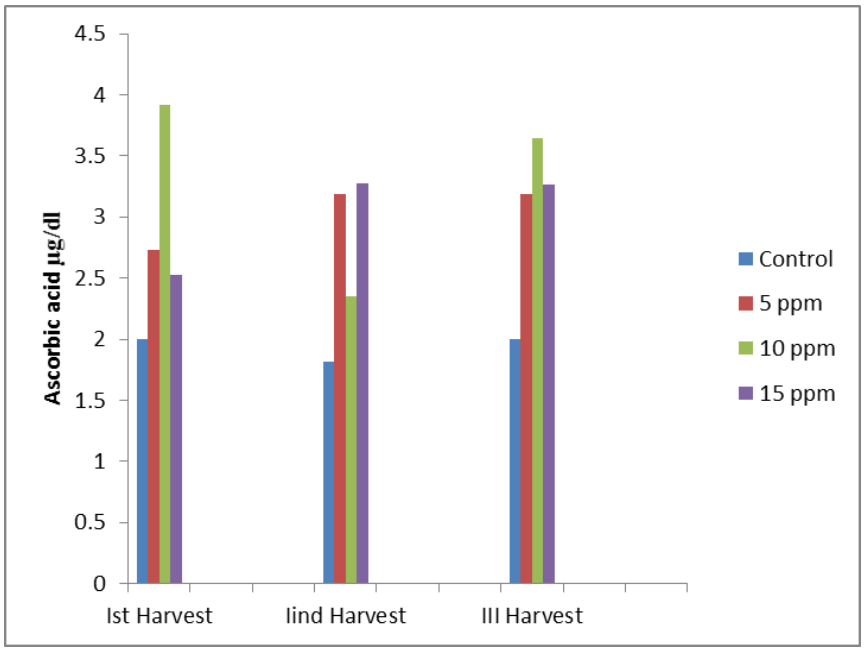

Fig 8: The effect of Cu on the ascorbic acid content (µ mol min^{-1} g^{-1}fw) in *Sorghum bicolor*. (On 15th, 30th and 45th days from day of germination)

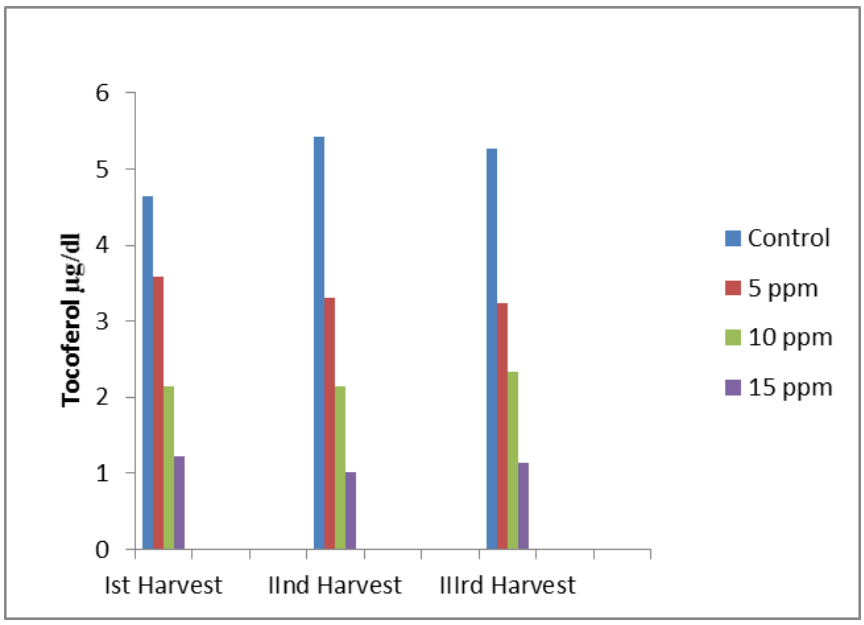

Fig 9: The effect of Cu on theα- tocopherol content (µ mol min^{-1} g^{-1}fw) in *Sorghum bicolor* leaves (on 15th, 30th and 45th days from day of germination)

4. Summary and Conclusion

The results of the present study are summarised as follows: Effect of copper on various phytochemical in *Sorghum bicolor* were examined at different periods of growth. The *sorghum bicolour* were grown in pots with Hoagland solution as a nutrient supplement and were treated with 0, 5.0ppm, 10.0ppm and 15.0ppm copper as copper sulfate ($CuSO_4.5H_20$).

The maximum decreases of 55.68%, 64.12% and 70.06% was observed at 15ppm after days of exposure period respectively. However, at days of exposure period, the chlorophyll a,b total and chlorophyll content was also decrease with increase in concentration of Cu. The maximum decrease of 51.36%, 52.29% and 55.71% was observed at 15.0 ppm after 30days of exposure as compared to control. 1st, 2nd, and 3rd harvest highest increase were obtained in 15.0 ppm Cu treatment *i.e.* 5.39%, 17.62% and 17.6% respectively in the comparison of control. Total phenol content in the leaves showed reduction with increasing cu concentration as compared to control at days. The maximum decrease of 59.4%, and 59.3% for 1st, and 2nd harvest respectively at 15ppm concentration of cu was observed at days as compared to control. Cardiac glycosides the maximum decrease of 51.36%, and 69.3% for 1st, and 2nd harvest respectively at 15ppm concentration of Cu was observed at days as compared to control. saponin in the leaves showed reduction with increasing cu concentration as compared to control at days. The maximum decrease of 52.29%, and 50.46% for 1st, and 2nd harvest respectively at 15ppm concentration of cu was observed at days as compared to control.

Tannin in the leaves showed reduction with increasing cu concentration as compared to control. The maximum decrease of 47.80%, a 52.87% and 38.69% for 1st, 2nd, and 3rd harvest respectively at 15ppm concentration of cu was observed at

days as compared to control. Amount of Flavonoid in the leaves showed reduction with increasing cu concentration as compared to control at days. The maximum decrease of 65.27%, and 65.35% for 1st, 3nd and harvest respectively at 15ppm concentration of cu was observed at days as compared to control. Maximum increased at 10ppm cu concentration in all the harvest as compared to their respective controls.

Ascorbic acid activity in the leaves showed maximum increase of 95.75% and 82% for I and III harvest respectively at 10ppm and 79.94% for 2nd harvest at 15ppm concentration of cu was observed at days as compared to control. Tocopherol activity in the leaves showed maximum increase of 73.69%, 81.34% and 78.29% for 1st, 2nd and 3nd harvest respectively at 15.0 ppm concentration of Cu was observed at days as compared to control. On the basis of above investigation it can be concluded that there is an effect of Cu. In higher plants, antioxidants are sufficient to prevent the biological damage up to more extent which is mediated by reactive oxygen species (ROS), which keep the deleterious reaction to a minimum. In addition these antioxidants in plants seem to bind metal in a form that renders metal harmless. The coordinated increase in the activity of enzymatic antioxidants renders tolerance to plant at lower metalloid concentration and initial period of exposure.

5. Acknowledgement

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6. References

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