

Changes in Growth and Photosynthetic Attributes of *Zea Mays* Cultivars (Fh 810 & Yousaf Wala) In Response to Copper Stress

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Abstract: The present study was carried out to investigate the effect of copper stress on FH 810 and Yousaf wala cultivars of *Zea mays*. From the study it is cleared that high concentrations of copper had negative effect on growth and photosynthetic attributes of both cultivars, as growth of plant decreased with increasing copper levels, same is the case with chlorophyll and carotenoid contents. FH 810 was more tolerant to copper stress than Yousaf wala cultivar. So, it is recommended that at high copper stress levels cultivation of FH 810 would be promoted than other one.

Key Words: Copper, *Zea mays*, growth and pigments

I. Introduction

Metal levels in the soil ranges from 1 mg/Kg-100 mg/Kg due to anthropogenic activities and geological foundation of soil [1]. Excess amount of cadmium, nickel, chromium, copper and zinc in soil caused deterioration of aquatic and terrestrial ecosystems [2 & 3]. With passage of time the release of heavy metals reached metric tons of 22,000 (Cd), 939,000 (Cu), 783,000 (Pb) and 1,350,000 (Zn) [4]. Sinha *et al.*, [5] suggested that Zinc, lead and copper is toxic to man health and caused serious problems in environment. These heavy metals are very toxic to aquatic animals and plants and also for human. Long duration exposure to heavy metals caused cancer of digestive tract and lungs and also the reason of vomiting, nausea. According to Saleemi [6] the contamination of water by heavy metal is the most invasive issue of the present and future time. As copper is also a heavy metal and act as a pollutant if present in large amount but it is necessary for plant development as it is a important micronutrient [7 & 8]. Copper actions are involved in lignifications because it is the integral component of many enzymes [8]. The Copper in ionic form is fundamental part of enzymatic activity because of its major role in plant respiration and photosynthesis [9]. Copper is included in the pollutant of air and soil if its limit exceed the standard values [10]. As copper is an essential micronutrient but it is dangerous in excess. Therefore, copper would be supplied in such amounts or insoluble forms to avoid its toxicity.

Zea mays L. (corn or maize) is the most important field crop and used as staple food in many countries and it has no wild existence. Vast diversity and numerous cultivars of maiz allowed it to adopt itself according to changing environmental conditions [11]. Its fruit i.e. corn contains carbohydrates, proteins, oils and minerals [12].

Keeping in view the above situation the present work was carried out using *Zea mays* cultivars to find out as copper is dangerous for *Zea mays* at high concentrations and to demonstrate the effect of copper on growth and photosynthetic attributes.

II. Materials And Methods

The experimental was consisted of total six treatments viz. a control and five treated one in each case (T₀ control, T₁ 5 mM/L, T₂ 10 mM/L, T₃ 20 mM/L, T₄ 40 mM/L, T₅ 80 mM/L) and two *Zea mays* cultivars (FH 810 & Yousaf wala). Each treatment was replicated three times and total seventy two pots were utilized in the experiment. After sowing, within four or five days cotyledon leaves were emerging out naturally. Two days after germinations of first green leaves, the first treatment was given to the plants to check the response of maize towards copper toxicity. After the preparation of different concentrations of solutions, 300 ml of each treatment solution was supplied to the treated plants. This dose was given to the plants once a week. Control plants (T₀) receive similar quantity of tap water. Plants were observed to execute the impact of the heavy metal. The control plants were healthy, lush green and showed normal growth while the treated plants exhibited stunted growth, pale green leaves, chlorosis, necrosis, wilting, etc. First destructive harvest of four-week old plants was carried out after giving four treatments and second destructive harvest was carried out after eight weeks. The study of different parameters like growth parameters, chlorophyll (a & b) and carotenoids determination was also conducted at each harvest.

Photosynthetic pigments

Chlorophyll “a” and “b” were determined by following Arnon [13]. 0.2 g of fresh leaves were extracted in 80% acetone over night and than centrifuged at 10,000xg for five minutes. Supernatant absorbance was noted at 480, 663 and 645 nm by using spectrophotometer (Hitachi-220 Japan). The chlorophyll contents were determined by using following formula:

$\text{Chl a (mg/g F. W)} = \frac{12.7 (\text{OD } 663) - 2.69 (\text{OD } 645) V}{1000 (W)}$

The carotenoids were calculated by the following formulae given by Krikk and Allen [14].

$\text{Chl b (mg/g F. W)} = \frac{22.9 (\text{OD } 645) - 4.68 (\text{OD } 663) V}{1000 (W)}$

$\text{Carotenoids (mg/g F. W)} = \text{OD } 480 + 0.114 (\text{OD } 663) - 0.638 (\text{OD } 645)$

Where, V = volume of the extract (mL), W = weight of the fresh leaf tissue (g) and OD =optical density

III. Results

The effect of different levels of copper T₀ (control), T₁ (5 mM/L), T₂ (10 mM/L), T₃ (20 MM/L), T₄ (40 MM/L), T₅ (80 mM/L) on the growth of root length had been presented in Figure 1 (A). The data in the figure showed that copper caused remarkable reduction in the root length of both maize cultivars and showed a decreasing trend towards different copper treatments. During harvest-I, the maximum reduction (52%) was observed in the Yousaf wala cultivar at 80 mM/L copper level than that observed for FH 810 cultivar. Similar results were observed at harvest-II that the maximum reduction (62.48%) was observed in Yousaf wala than for FH 810 cultivar. The data showed that FH 810 had better growth under various copper treatments as compared to Yousaf wala. Data in Figure 1 (B) revealed the effect of different levels of copper on the growth of shoot length during both harvests. The data showed that copper caused considerable reduction in the shoot length of both maize cultivars and showed a decreasing trend towards different copper treatments. It was found that application of different Cu treatments to the potted plants proved very effective although least effects were observed on T1. The maximum reduction (47.6% & 58.9%) was observed in the Yousaf wala than for FH 810 at both harvests.

The results revealed that decreased in root fresh weigh was more pronounced with increasing copper levels (Figure 1C). During harvest-I & harvest-II the more reduction (39.0 & 47.3 %) in shoot fresh weight was note in Yousaf wala cultivar as compared to FH 810. In the case of shoot fresh weight during 1st harvest, the maximum reduction (30.52%) was observed in the second cultivar (Yousaf wala) at T₅ treatment of copper than that observed for FH 810. Same is the case at harvest-II (Figure 1 D).

Whereas, in the case of root and shoot dry weight similar trend was observed as shown by fresh weight of both. In the case of root dry weight the maximum reduction (38.05%) was observed in the yousaf wala (Figure 1 E). But for shoot dry weight the maximum reduction (49.8%) was observed again in Yousaf wala cultivar (Figure 1 F).

Copper had toxic effect on photosynthetic attributes of both Zea mays cultivars. As concentration of copper increased there is markedly decreased in chlorophyll a, b and carotenoids contents of leaves. The amount of cholorophyll a and b contents decreased very sharply as compared to carotenoid contents. Figures 2 (A,B & C) clearly showed the decreased in photosynthetic pigments with increased in copper concentrarion. FH 810 was become more tolerant to copper stress than Yous wala cultivar of Zea mays. The reduction in chlorophyll a (5.23%-52.63%) and chlorophyll b (7.69%-55%) contents was observed in both cultivars. The data revealing the percentage reduction was presented in Table 1.

IV. Discussion

Copper is dominant pollutant of air and agricultural soil. Copper required in trace amount in various metabolic processes but it is toxic at higher concentrations. [15]. In present study, a preliminary investigation into the effect of copper on the morphological and photosynthetic attributes was carried out. Concentration of copper used in this study included: 5 mM/L (T1), 10 mM/L (T2), 20 mM/L (T3), 40 mM/L (T4), 80 mM/L (T5) along with control (T0). All concentration of copper affected various attributes of both cultivars of *Zea mays* ranging from slightest to moderate and severe. Vegetative growth and related parameters of *Zea mays* plant were affected by different concentration of copper (5, 10, 20, 40, 80 mM/L). These effects were highly pronounced in T1-T5 Cu-treated plants as compare with control. At root length per plant (8-62%), shoot length per plant (12-58%), root fresh weight per plant (7-47%), shoot fresh weight per plant (6-14%), root dry weight per plant (8-47%), shoot dry weight per plant (5-49 %), chlorophyll a (5.23%-52.63%) and chlorophyll b (7.69%-55%) and carotenoid contents (8.36%-57.13%).

Our findings correlate with the findings of Liu et al, [16] who investigated the effect of copper sulphate on the growth rates, Cu uptake and its accumulation by roots and shoots of maize. Different concentration of CuSO₄ was used and observed that root length, fresh & dry weight decrease with increasing concentration of copper. Amount of Cu in root of controlled plants was less than root of Cu-treated plants. However, shoot had less amount of Cu than that of root.

Similarly, in this present study it was investigated that plant root length, root dry weight and plant total dry weight decreased with the increased Cu concentration, which is accordance with the studies of Zheng et al, [17] who observed the effect of copper in three ornamental plants and conclude that a significant reduction in plant root length, root dry weight and total dry weight of plant occurred with increasing Cu concentration. This change in root morphology with copper toxicity and elongation of root inhibited by the application of copper, due to decrease in Indole Acetic Acid oxidase activity in roots [18]. Hendry [19] studied the effect of copper toxicity in maize plants and found that Cu treatment in maize completely inhibited the root growth. Agarwal *et al.*, (1987) concluded that decrease root growth is due to the reduction in cell division resulted because of inhibitory effect of Cu. High level of Cu inhibits root growth before shoot production because roots are in environment where copper is in excess [20]. But reduction in shoot growth was directly related to heavy metal concentration in many plant species [9& 21-22]. The photosynthetic pigments have ability to limit the photosynthetic rate. From the results it was concluded that carotenoids decreased less as compared to chlorophyll. The decreased in both contents is due to the oxidative stress of copper or other environmental factors [19]. A reduction of pigment contents with more and more heavy metals was also suggested by Bogoea [23]. The decreased in chlorophyll contents may be due to damaging of chlorophyll molecules and inhibition in synthesis of chlorophyll [24]. So, on the basis of present work it was concluded that copper is more toxic for plants if its limit exceeds the standard values.

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Table 1: Percent decrease in growth and photosynthetic attributes of two Zea mays cultivars (FH-810 & Yousafwala) under copper stress at harvest-I & II.

Attributes	Harvests	Cultivars	Treatments / %age reduction				
			T1 (5 mM/L)	T2 (10 mM/L)	T3 (20 mM/L)	T4 (40 mM/L)	T5 (80 mM/L)
Root length (cm)	H-I	FH 810	8.61	17.04	25.46	33.24	40.37
		Yousaf wala	14.52	25.8	35.47	44.78	52.11
	H-II	FH 810	14.71	27.04	36.44	41.44	49.92
		Yousaf wala	17.4	33.73	44.35	54.68	62.48
Shoot length (cm)	H-I	FH 810	12.6	22.1	30.4	38.2	44.9
		Yousaf wala	8.8	24.7	33.6	42	47.6
	H-II	FH 810	10.07	23.2	34.1	43.4	51.7
		Yousaf wala	12.3	24.7	39	50	58.9
Root fresh weight (g)	H-I	FH 810	7.09	13.9	18	23.3	28.1
		Yousaf wala	7.7	14.9	22.8	31	39
	H-II	FH 810	4.7	12.4	21.4	29.6	38.3
		Yousaf wala	6.4	17.2	29.4	36.9	47.3
Shoot fresh weight (g)	H-I	FH 810	6.46	11.58	17.62	21.89	27.21
		Yousaf wala	7.44	12.6	18.01	23.3	30.52
	H-II	FH 810	7.5	13.9	19.94	28.3	35.54
		Yousaf wala	7.96	15.43	21.38	30.55	41.62
Root dry weight (g)	H-I	FH 810	8.26	13.8	20	25.1	30
		Yousaf wala	8.6	16.8	23.7	31.5	38
	H-II	FH 810	8.71	16.5	25.1	33.6	40.5
		Yousaf wala	10.2	17.9	30.7	36.7	47.4
Shoot dry weight (g)	H-I	FH 810	5.07	9.81	15.5	23.9	29.1
		Yousaf wala	5.92	14.1	24.4	31.8	39.5
	H-II	FH 810	10.46	19.7	26.01	34.1	40.5
		Yousaf wala	9.8	21.6	34.7	43.9	49.8
Chlorophyll "a" (mg/g F.W)	H-I	FH 810	5.23	12.79	21.15	28.48	34.88
		Yousaf wala	9.15	16.99	26.79	33.98	39.21
	H-II	FH 810	12.06	19.59	29.14	36.68	47.23
		Yousaf wala	15.78	27.89	38.42	46.31	52.63
Chlorophyll "b" (mg/g F. W)	H-I	FH 810	7.69	14.17	22.26	28.34	35.22
		Yousaf wala	10.04	17.9	26.2	33.18	38.86
	H-II	FH 810	12.88	24.06	32.2	38.98	47.79
		Yousaf wala	15.41	27.5	38.75	45.83	55
Carotenoids (mg/g F.W)	H-I	FH 810	8.36	15.3	22.2	28.5	35.5
		Yousaf wala	10.5	18.2	25.8	32.5	39.7
	H-II	FH 810	9.88	19.09	26.08	34.72	44.37
		Yousaf wala	9.31	19.27	32.01	45.47	57.13

Figure 1: Decrease in (A) root length (cm), (B) shoot length (cm), (C & D) root and shoot fresh weight, (E & F) root and shoot dry weight of two Zea mays cultivars (FH-810 & Yousafwala) under copper stress at harvest-I & II.

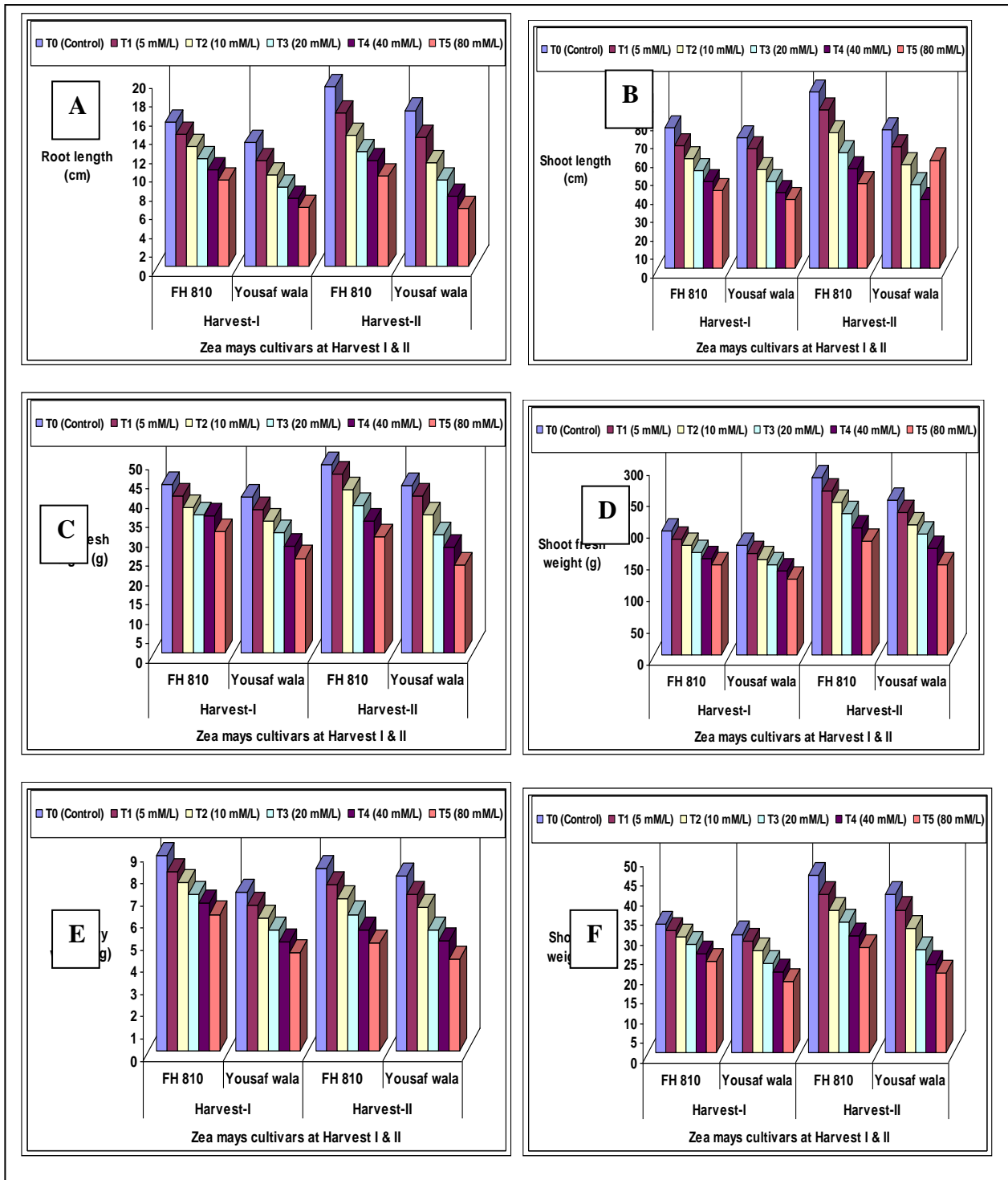


Figure 2: Decrease in (A) Chlorophyll “a” (B) Chlorophyll “b” and (C) Carotenoid contents of two Zea mays cultivars (FH-810 & Yousafwala) under copper stress at harvest-I & II.

Figure 1: Decrease in (A) Chlorophyll “a”, (B) Chlorophyll “b” and (C) carotenoid (mg/g F.W) contents of two Zea mays cultivars (FH-810 & Yousafwala) under copper stress at harvest-I & II.

