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RESEARCH ARTICLE

PERFORMANCE OF TWO HYBRIDS OF *Helianthus annuus* L. (SUNFLOWER) UNDER THE STRESS OF HEAVY METALS i.e. ZINC AND COPPER

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ABSTRACT

A pot experiment was conducted in University of Agriculture Faisalabad to assess the effect of Cu and Zn uptake on morphological, physiological, biochemical and yield attributes of *Helianthus annuus* L.(Sunflower). Two hybrids FH-612 and FH-621 were used during this study. The experiment was laid out in a Completely Randomized Design (CRD) with four treatments and three replicates. After 20 days of germination the plants were subjected to different levels of $ZnCl_2$, $CuSO_4$ and with their combination. Three harvests were taken at an interval of seven days each to study the growth rate, morphological, physiological and biochemical attributes. At the maturity of crops final harvest was taken and yield attributes were recorded. Data of various attributes were statistically analyzed. It was observed that both the metals caused negative effect on growth rate as well as the other parameters studied. The effect was more pronounced in the metal combination treatment. Ultimately the yield was significantly reduced due to the effect of metals in both hybrids. However hybrid FH-612 revealed slight tolerance towards Cu and Zn toxicity as compared to its counterpart. FH- 621. Thus it was concluded that Cu and Zn affect the morphological, physiological and yield attributes of sunflower when applied in higher concentration.

KEYWORDS

Copper, Zinc, Sunflower, Gas exchange parameter, Chlorophyll pigments Achene Yield.

1. INTRODUCTION

The heavy metals which are enumerated as significant pollutants by the US Environmental Protection Agency has given a problem due to increasing urbanization and industrialization (Rai, 2007). The main environmental apprehension worldwide is water and soil pollution by toxic heavy metals (Larison et al., 2000). In the soil and water, an increase in heavy metals results in increased metal uptake by plants (Farooq et al., 2008). Crops grown in metal contaminated soil can accumulate metals in various food parts which are then transferred to human body and cause many diseases (Kumar and Clark, 1991).

Heavy metals cannot be destroyed or despoiled. They bio-accumulate over a period and enter the body structure through water, air and food to a small level (Lenntech, 2004). Metals affect both metabolism and development of plants at high concentration. Heavy metals affect different physiological attributes and produce nutritional abnormalities, protein and chlorophyll degradation in many flora (Ahmad et al., 2011). These phytotoxic effects of heavy metals depend on pH of soil plant species, metal absorption capacity and other factors in soil (Kumar et al., 2004). Heavy metals polluted soils can be cleaned by different methods which are very expensive and persistent consisting mainly of quarry, discarding of harmful wastes, stabilization of the soil with cement or alike materials (Srivastava and Goyal, 2010). Phytoremediation is the best expertise to take out pollutants from the environment (Srivastava and Goyal, 2010). Phyto-extraction of polluted soils is also well thought-out to be environmentally friendly and efficient (Lone et al., 2010).

Although zinc and copper are vital nutrients for plants but at higher levels, become toxic. Zinc is absorbed in the form of Zn^{+2} through roots from soil solution. It is an important micronutrient and acts as a protein in many essential biochemical pathways (Alloway, 2008). Zinc in excess amount generates Reactive Oxygen Species (ROS) and /or dislocates other metals in proteins from their active sites. Its toxic amount also induces chlorosis in young leaves, reduces tissue water content and changes concentrations of the Mg and P in plant tissues (Marschner, 1995). Zinc deficiency leads up to 50% reduction in crop growth without the appearance of visual symptoms (Murthy, 2011) and also leads to decrease in synthesis of protein (Marschner, 1995) due to RNA degradation (Cakmak et al., 1989). Cu is a main heavy metal pollutant that exists as Cu^{2+} and Cu^{+}. In many enzymes, Cu ions act as cofactors (Halliwell and Gutteridge, 1984). If Cu concentration is in excess amount then inhibits seed germination and plant growth, interferes with photosystem action and induces chlorophyll deprivation (Caspi et al., 1999). In non-tolerant plants, excess amount of Cu may damage root cell membranes and inhibit elongation of root (Wainwright and Woolhouse, 1977).

Zengin and Kirbag investigated the effect of Cu chloride ($CuCl_2$) on sunflower (*Helianthus annuus* L.) seedlings and observed that as Cu concentration increased, the level of total protein and chlorophyll (a+b) unusually decreased (Zengin and Kirbag, 2007). In other studies, authors observed the high absorption of the Cu in Water Hyacinth (*Eichhornia crassipes*), Sunflower (*Helianthus annuus* L.), and Indian Mustard (*Brassica juncea*) plants as compared to lead (Patel et al., 2013). Accumulation of the Cu in sunflower was more as compared to other two plants.

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Helianthus annuus L. (Sunflower) belongs to the family Asteraceae, widely cultivated for vegetable oil and as a staple food in many areas of the world. The total area under cultivation in Pakistan is 506, 000 ha and production from this area is 755, 000 tons (seed) with average yield of 1.6 t ha^{-1} (Anon, 2009). The objective of this research was to check the effect of heavy metals i.e. Zinc and Copper on hybrids of *Helianthus annuus* L. i.e. FH-612 and FH-621 regarding physiological, morphological, biochemical and yield responses.

2. Materials and Methods

An experiment was conducted under natural environmental conditions in Old Botanical Garden, University of Agriculture, Faisalabad, during the year 2014-2015 to make a comparison between two hybrids of sunflower i.e. FH-612 and FH-621 to the heavy metals i.e. Copper and Zinc. Seeds of both varieties were sown in different pots under the same environmental conditions and applied the heavy metals after 20 days of their germination. For each hybrid, there were four treatments each replicated thrice. In addition to control treatments consisted of 75 ppm copper, 100 ppm zinc and 75 ppm copper + 100 ppm zinc. The experiment was laid out in a completely randomized design (CRD) with two factorial arrangements.

Growth rates for parameters such as root length, shoot length, number of leaves, fresh and dry weights were measured. For growth studies three harvests were taken at an interval of seven days each after the treatment application. Each time 3 plants were uprooted from each treatment of a hybrid. Spectrophotometer was used to determine the chlorophyll content of each plant and their data was recorded. IRGA (Infra-Red Gas Analyzer) was used to measure physiological parameters including Transpiration rate (E), Photosynthetic rate (A), Water use efficiency (A/E), Stomatal conductance (Gs) and Sub stomatal carbon dioxide concentration (Ci). Ion analysis was performed per method prescribed by digestion. Yield parameters were recorded at maturity of the crop.

All the collected data were statistically analyzed using analysis of variance technique based on two factors completely randomized design (CRD) using COSTAT computer package (Cohort software Beckely California), while treatment means were compared by Duncan's Multiple Range test (Steel and Torrie, 1986).

3. Results and Discussion

Growth analysis studies revealed that the growth rate of all organs in both hybrids got decreased due to Cu and Zn stress. Elongation of shoot and root got showed down; decrease occurred in leaf area and consequently biomass was negatively affected in treated plants as compared to control. The deteriorating effect of metals was comparatively more during the first harvest interval as compared to second interval. The dry biomass of plants was decreased due to application of Cu, Zn and Cu+Zn in the first harvest interval while in second harvest interval the treated plants indicated slight improvement. It was noted that Zn caused greater detrimental effect than the Cu while their combine application was more harmful than their individual effect. Gas exchange parameters were negatively influenced in sunflower hybrids by both the metals Cu and Zn. Net assimilation rate, transpiration rate, stomatal conductance and substomatal CO_2 concentration got decreased under metal stress. Significant reduction was calculated for assimilation rate by metal stress as compared to control.

The treatments receiving individual Cu and Zn applications differed non-significantly from one another. Combined metal treatment caused severe effect than individual metals differing significantly from individual application treatment as well as control plants. The same trend was observed for all gas exchange parameters in sunflower crop. The hybrids×treatment interaction was mostly non-significant in these parameters indicating similar behavior of both hybrids to metal stress.

Photosynthetic pigments (Table 1) were adversely affected in sunflower crop by Cu and Zn applications. Significant reduction was calculated by both individual and combined metal applications as compared to control plants. Combined metal application proved more harsh for chlorophyll contents than individual metal application. None of the two hybrids exhibited tolerance for these metals. Sodium, Calcium, Potassium and phosphorus were determined in roots and shoots of the sunflower crop after metal stress and were calculated to get disturbed (Figure 2). The metals exerted negative effect on yield parameters as well. Capitulum diameter got reduced by all treatments exhibiting significant differences from control,100 achene wt. was noted to be decreased in treated plants, but this decrease was non-significant. All these negative effects got reflected in achene yield that was reduced significantly.

Maximum reduction of 35% was calculated in achene yield by combined metal application. The difference between two hybrids was non-significant showing similar response of both towards heavy metal application. It is a common fact that heavy metals in soil exert toxic effects on plants, when present in excess amounts than permissible limits. Zinc and copper have been included amongst the toxic metals since long (Roseby et al., 1998). Cu caused negative effects in various crops (Caypers et al., 2011). Similarly, zinc has been reported for effects in numerous studies. It reduced growth and yield in Saccharum officinarum and other crops (Chatterjee et al.,1998; Ozdener and Aydin, 2010). These metal disturb synthesis of photosynthetic pigments or cause disturbances in their action. The level of these pigments has been reported to be reduced metabolism of plants gets altered (Ajoykumar and Sreya, 2018; Iqbal et al., 2015). These effects result in decrease of economical yield of the crops.

Table 1: Effect of Cu, and Zn stress on photosynthetic pigments and gas exchange parameters in sunflower hybrids

	Mean Square	F.Value	C	Cu	Zn	Cu+Zn
Assimilation Rate	30.489	18.7743	26.99	24.51	23.04	20.05
Transpiration rate	0.278	15.2685	2.96	2.59	2.56	2.47
Stomatal conductance	0.001	3.5862	0.14	0.12	0.11	0.09
Sub-Stomatal CO2 conc.	5.397	62.208	323.26	309.26	271.98	255.67
Chlorophyll "a"	2.796	3.6973	0.009	0.008	0.08	0.07
Chlorophyll b	3.193	2.0545	0.002	0.001	0.001	0.001
Carotenoids	0.003	0.4967	0.417	0.403	0.331	0.367
Total Chlorophyll	9.939	4.0254	0.012	0.010	0.009	0.009

Table 2: Yield Parameters of sunflower as affected by Cu and Zn treatments

Parameter	Mean square	F-value	Treatment means C	Cu	Zn	Cu+Zn
Capitulum diam.(cm)	6.373	17.693	5.190	4.05	3.30	2.83
100 achene wt.(g)	0.087	1.3248ns	2.04	1.96	1.84	1.77
Achene yield/plant (g)	1.502	6.9095	3.03	2.60	2.05	1.97

Table 3: Effect of Zn and Cu on relative increase in shoot length (cm) of two hybrids of *Helianthus annuus* L. (Sunflower).

FH-612

Harvest Interval	T_0	T_1	T_2	T_3
1st Harvest Interval	9.8	8.7	8.3	7.3
% difference		11.2%	15.3%	25.5%
2nd Harvest Interval	7.9	6.8	6.5	5.8
% difference		13.92%	17.72%	26%

FH-621

Harvest Interval	T_0	T_1	T_2	T_3
1st Harvest Interval	8.5	7.9	7.3	6.2
% difference		7.05%	14.1%	27.1%
2nd Harvest Interval	7.7	6.8	6.3	5.9
% difference		11.68%	18.18%	23.37%

Table 3.1: Effect of Zn and Cu on relative increase in shoot dry weight (g) of two hybrids of *Helianthus annuus* L. (Sunflower).

FH-612

Harvest Interval	T_0	T_1	T_2	T_3
1st Harvest Interval	1.8	1.5	1.4	1.2
% difference		16.67%	22.22%	33.33%
2nd Harvest Interval	1.6	1.4	1.3	1.1
% difference		12.5%	18.75%	31.25%

FH-621				
Harvest Interval	T_0	T_1	T_2	T_3
1st Harvest Interval	1.5	1.4	1.3	1.1
% difference		6.66%	13.33%	26.66%
2nd Harvest Interval	1.4	1.3	1.1	1.0
% difference		7.14%	21.42%	28.57%

Table 3.2: Effect of Zn and Cu on relative increase in root dry weight (g) of two hybrids of *Helianthus annuus* L. (Sunflower).

FH-612				
Harvest Interval	T_0	T_1	T_2	T_3
1st Harvest Interval	1.5	1.4	1.2	1.1
% difference		6.67%	20%	26.67%
2nd Harvest Interval	1.3	1.2	1.1	0.9
% difference		7.69%	15.38%	30.77%

FH-621				
Harvest Interval	T_0	T_1	T_2	T_3
1st Harvest Interval	1.2	1.1	0.9	0.8
% difference		8.33%	25%	33.33%
2nd Harvest Interval	1.1	1.0	0.9	0.8
% difference		9.09%	18.18%	27.27%

Table 3.3: Effect of Zn and Cu on relative increase in leaf area (g) of two hybrids of *Helianthus annuus* L. (Sunflower).

FH-612				
Harvest Interval	T_0	T_1	T_2	T_3
1st Harvest Interval	3.5	3.1	3.0	2.8
% difference		11.43%	14.28%	20%
2nd Harvest Interval	3.3	2.8	2.6	2.4
% difference		15.15%	21.21%	27.27%

FH-621				
Harvest Interval	T_0	T_1	T_2	T_3
1st Harvest Interval	2.4	2.1	1.9	1.7
% difference		12.5%	20.83%	29.16%
2nd Harvest Interval	2.0	1.7	1.5	1.4
% difference		15%	25%	30%

3.1 Calcium contents in roots

	T0	T1	T2	T3
FH- 612	11.333	11.667	10.667	9.000
FH- 621	11.000	11.333	13.000	11.000

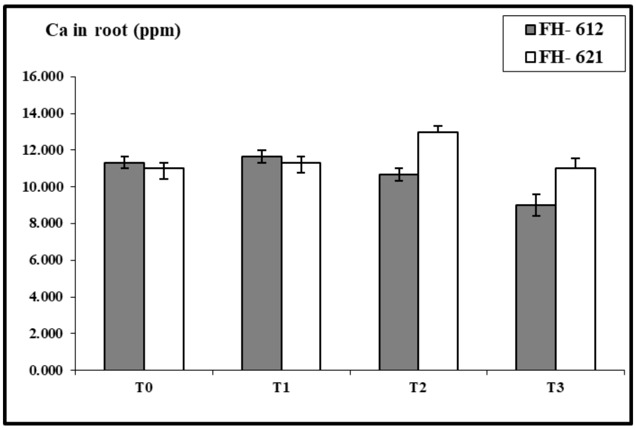
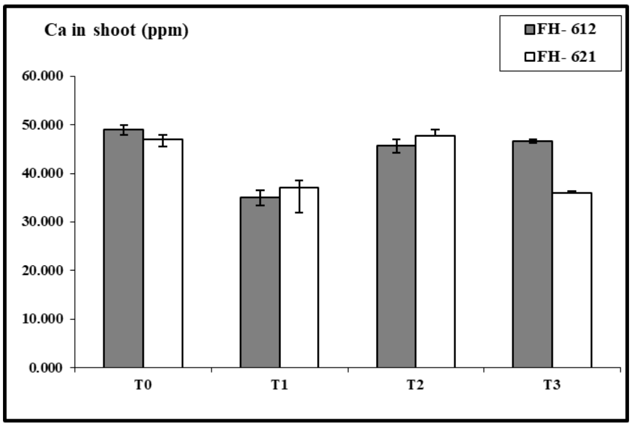


Figure 1: Growth analysis of sunflower hybrids under Cu and Zn stress.

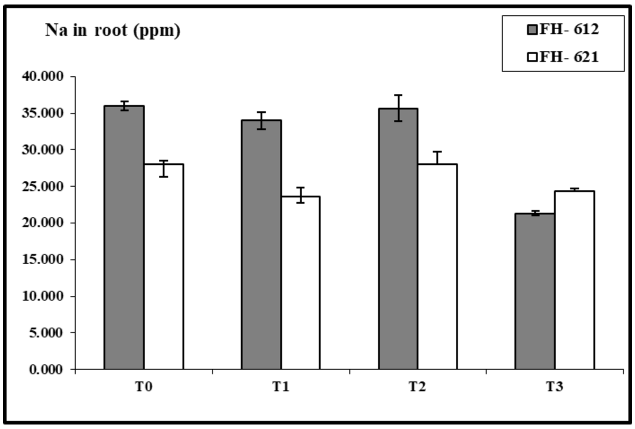
3.2 Ca contents in shoot

	T0	T1	T2	T3
FH- 612	49.000	35.000	45.667	46.667
FH- 621	47.000	37.000	47.667	36.000



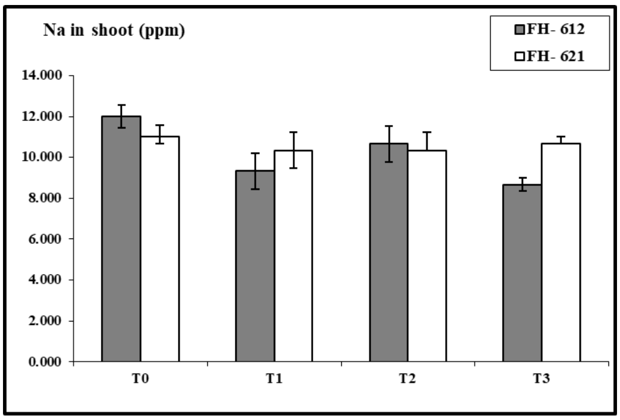
3.3 Sodium contents in roots

	T0	T1	T2	T3
FH- 612	36.000	34.000	35.667	21.333
FH- 621	28.000	23.667	28.000	24.333



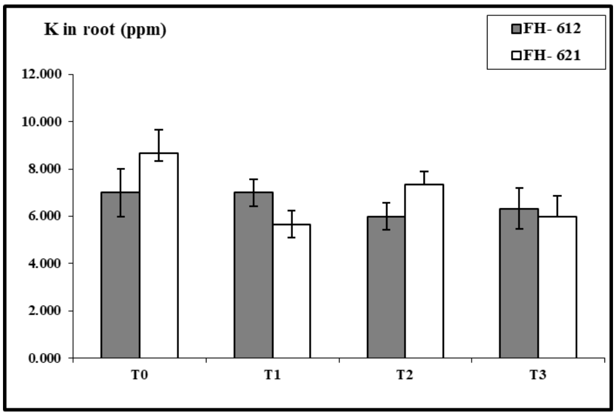
3.4 Sodium contents in Shoots

	T0	T1	T2	T3
FH- 612	12.000	9.333	10.667	8.667
FH- 621	11.000	10.333	10.333	10.667



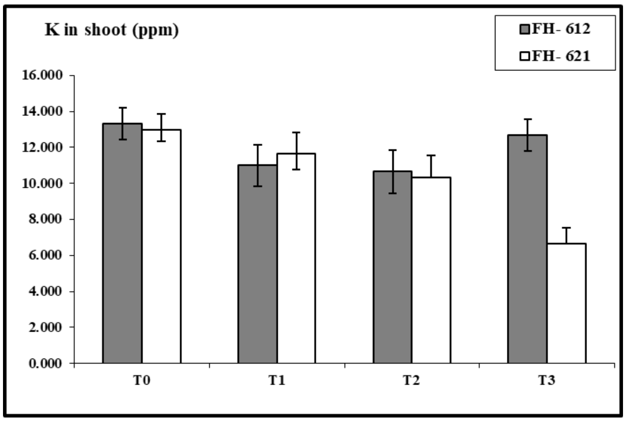
3.5 K contents in root

	T0	T1	T2	T3
FH- 612	7.000	7.000	6.000	6.333
FH- 621	8.667	5.667	7.333	6.000



3.6 K contents in shoot

	T0	T1	T2	T3
FH- 612	13.333	11.000	10.667	12.667
FH- 621	13.000	11.667	10.333	6.667



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