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Physiological, anatomical and morphological studies of different parts of sunflower

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Abstract

Sunflower (*Helianthus annuus* L.) is an important ornamental plant and god source of oil, widely accepted as potential promising plant for phytoremediation. This study was carried out to evaluate the impact of cadmium stress on growth, chlorophyll contents, of sunflower plants two varieties V1 (F1-682) and V2 (FH-626). Sun flower plants (*Helianthus annuus* L.) were exposed to different concentration (0, 10, 20mM) $CdCl_2$ to evaluate the effects of cadmium (Cd) exposure on sunflower (*Helianthus annuus* L.). Growth parameters (shoot, root length, fresh and dry weights, chlorophyll contents) were determined and results compared with two parameters associated with photosynthesis degradation, chlorophyll content. The height of the shoots of plants was measured just before harvesting and number of leaves were counted to verify possible disorders in their growth due to exposure to cadmium. Exposure to Cd significantly decreased growth by decreasing shoot and root lengths and their fresh and dry weight. From Cd-lower and Cd-higher treatments showed necrosis at the base of their stems, as well as foliar chlorosis in those plants.

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Introduction

Cadmium (Cd) is a highly toxic pollutant released into the environment by both anthropogenic and natural resources. The accumulation of Cd in biotic systems as a consequence of human activities is becoming a major environmental problem. The application of sewage sludge, city waste, and Cd-containing fertilizers causes the increase of Cd content in soils.Cadmium may reduce plant growth by inhibition of chlorophyll synthesis (Stobartetal *et al.*, 2003).

The presence of excessive amount of Cd in soil causes many toxic symptoms in plants, such as reduction of growth, especially root growth, disturbances in mineral nutrition and carbohydrate metabolism and may therefore strongly reduce bioma Its presence in the soil, including agricultural lands, is considered a serious environmental issue mainly due to its entry in the food human chain with dangerous effects on living organisms (Weigel and Jager, 1985).

Sunflower (*Helianthus annuus*) is an important ornamental plant and good source of vegetable oil use for commercial and industrial purpose also considered as an important component of phytoremediation of organic & inorganic source soil contamination (Adesodun *et al.,* 2010).

The study and evaluation of metals and metalloids in different biological systems is an important scientific field when focusing on its importance to many areas. This task make possible a bridge between research of different background, producing more coherent and effective results.

Generally, plants can tolerate low levels of Cd in soils, but high levels can cause toxicity symptoms, including growth reduction, leaf rolling and chlorosis. Cadmium, is accumulated in sunflower leaves, where the defense mechanism developed by the plant species to minimize the phyto- toxic effect caused by excess of ions in their tissues is selenium metabolization in to volatile species, allowing their elimination through oliar transpiration. These facts provide evidence that accumulation of metals or metalloids in the roots r shoots is associated with metabolic pathways outlined by the plant in an attempt to establish metabolism homeostasis (Dhainbani *et al.*, 2013).

The aims of this study will be investigating the physiological, anatomical and morphological studies of different parts of sunflower such as root length, shoot length, fresh weight, dry weight, chlorophyll concentrations such as chlorophyll a, b and carotenoids. This study involved the effect of cadmium that is heavy metal on growth of different parts of sunflower plant.

Materials and methods

Research is conducted to see the effect of cadmium (Cd) salt on growth of sunflower plant. The experiment show that heavy metal cause bad effect on plants of sunflower on its germination ,growth production parameters like leaf area root length, shoot length lack of chlorophyll all effects are due to stress of heavy metal Cd.

Study Design

To conduct this experiment or to check the effect of heavy metals stress on plant of sunflower in the Old Botanical Garden of University of Agriculture Faisalabad. The plants are keep under full observation in day length of 12 hours. Thus, the influence of cadmium on sunflower plants (roots, Stems and leaves) was systematically evaluated through a comparative ionomic approach in this work. For this task, sunflower plants were grown at different irrigation conditions employing solutions with different concentrations of cadmium.

Treatment and Replications

Effect of heavy metals on growth and morphology of sunflower observe between 11-3-2019 to 16-3-2019 seeds were brought from Seed Lab of Horticulture, Department of Agriculture University Faisalabad, Pakistan. All experiments were carried out in Old Botanical Garden of UAF. All pots were arranged in CRD (Complete Randomized Design) all 18 pots were named as on the basis of variety and treatment.

The first row were named as (V1TOR1, V1TOR2, V1TOR3, V2TOR1, V2TOR2, V2TOR3,) them second row were named as (V1T1RI, V1TIR2, V1T1R3, V2T1R1, V2T1R2, V1T2R3). The 3rd row were named as (V1T2R1, V1T2R2, V1T2R3, V2T2R1, V2T2R2, and V2T2R3).

Sowing & Germination

The experiment were carried out on Sun flower (*Helianthus annuus*) two varieties V1 (F1-682) & V2 (FH-626). The growing media was soil which was air dried and homogenized .The total number of pots was 18 which are filled with growing media soil. The sowing was done 11-3-2019 in each pot total 5 seed were sown 3 pots were designed in completely randomized design and each treatment was replicated 6 times ,18 pots for 3 treatments in which 6 for normal treatment and 12 for Cadmium chloride salt treatment (TO, T1 Cd10, T2 Cd 20µm). The stress was applied after 2 weeks of germination of all seeds the all replicates were treated with simple tap water on daily basis. After sowing of seeds the germination start after one week of sowing the seedlings appear in the pots on 18-3-2019. The all seeds which were sowed start germination except few one.

Number of Treatments

After two weeks of germination cadmium stress is applied the treatment was applied on the basis of given series name. The first row were selected as control series which receive only simple tap water V1TOR1, V1TOR2, V1TOR3, V2TOR1, V2TOR2, V2TOR3,). The second were selected of Treatment 1 which is treated with Cd solution which was 10mM (V1T1RI, V1TIR2, V1T1R3, V2T1R1, V2T1R2, V1T2R3) .Third row for T2 which was Cd 20mM solution as (V1T2R1, V1T2R2, V1T2R3, V2T2R1, V2T2R, V2T2R3). The 2nd row treated with CdCl2 20 mm solution which is made by weight CdCl2 10µg and dissolve it litter simple tap water. 3rd replica which called second treatment it give 20µM solution of CdCl2 (The 10µM solution of CdCl2 is made by weight 20 mg salt and dissolve it 1 litter simple tap water.

TO= tap water

T1= Cadmium chloride 10mM solution

T2=Cadmium chloride 20mM solution

In 1st table the replicates of control series are shown which are treated with only simple tap water till harvesting. The 2nd table shows the replicates of second row in which both V1, V2 of sunflower included the stress is applied on them after 2 weeks of germination CdCl 10 mM solution. The 3rd table shows the all replicates of 3rd row which also contain V1, V2 of sunflower both varieties are treated equally with $CdCl_2$ 20mM solution after 2weeks of germination.

Harvesting

The plants were harvested carefully on 16-5-2019 after 16 days of stress application. Shoot and root are taken from harvested plants which are healthy two plants were selected from each pot they have healthy shoot and roots. Root FW and shoot FW are calculated root length also for root and shoot DW both are kept in incubator for one night then there DW weights are calculated.

Measurement & Analysis

Sunflower plants were harvested 8 weeks after planting. Before the harvest, fresh leaf samples were taken out from the youngest fully expanded leaf for MP, Chl, and Car analyses. The fresh weight (FW) and dry weight (DW) of shoot and root were separately measured. The shoot samples were weighed for FW, washed with running tap water, and rinsed with distilled water. The root samples were taken out carefully, removed the soil particles, rinsed with distilled water, measured the lengths, and weighed for FW. Also, they were dipped in CaCl2 solution (10 mM) for 10 minutes in order to eliminate adsorbed trace elements from the root surface and then rinsed three times with cold distilled water (Stold *et al.,* 2003). The shoot and root samples were dried at 70°C, DW of the samples were quickly measured and subsequently ground to powder for nutrient element analysis. The dried tissues were digested by dry-ashing method in a muffle furnace at 500°C for 6 hours and dissolved in 10 N HNO3 (Miller, 2004).

Photosynthetic pigments were measured in the youngest fully expanded fresh leaf samples before harvest. The fresh leaf samples (200mg) were cut into small pieces and were homogenized in 10 mL of acetone (90%, v/v). The extract was then filtered and the absorbance of the extract was measured at 663, 645, and 470nm using a spectrophotometer. The concentrations of Chl, and Car were calculated according to the formula given.

Photosynthetic pigments

Photosynthetic pigments were extracted from leaf samples (0.5g) in 80% acetone as described by Arnon (1949). The optical densities of the supernatant were recorded at 480, 645 and 663 nm against a blank containing acetone (80%).

Results and discussion

Analysis of variance of data for shoot length of sunflower FH682.FH626 were grown under different concentration of cadmium chloride ($CdCl_2$) A significant increase was observed in shoot length. Maximum decrease was observed when 20mM $CdCl_2$ applied as compared to control. Variety FH626 showed more shoot length as compared to Variety FH682. Analysis of variance of data for root length of sunflower FH682.FH626 were grown under different concentration of cadmium chloride ($CdCl_2$) A weighty increase was observed in root length. while maximum decrease was observed when 10,20mM $CdCl_2$ as applied. Sunflower performs better growth in control. Variety V2 FH626 showed more root length as compared to Variety v1 FH682. Analysis of variance of data for shoot fresh weight of sunflower variety FH682.FH626 were grown under different concentration of cadmium chloride ($CdCL_2$) A significant increase was observed in shoot fresh weight of control series as compared to 10,20mM treatment. Maximum decrease was observed when 10mM,20mM $CdCL_2$ was applied. Variety FH626 showed more shoot fresh weight as compared to Variety FH682.

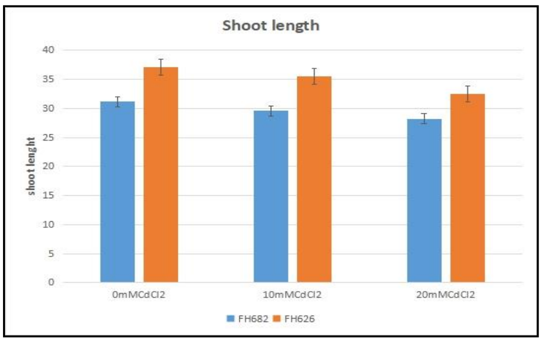

Fig. 1. Shoot length in FH682, FH626 Treatment (0,10,20)mM.

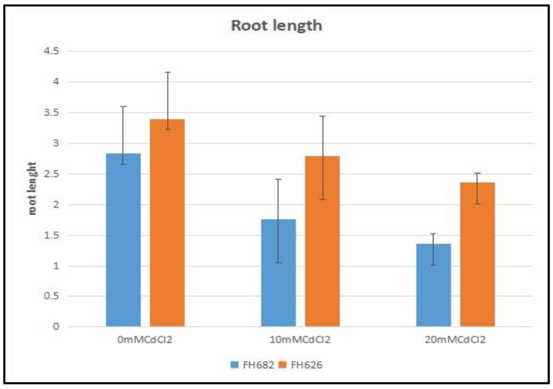

Fig. 2. Root length in in FH682, FH626 Treatment (0,10, 20)mM.

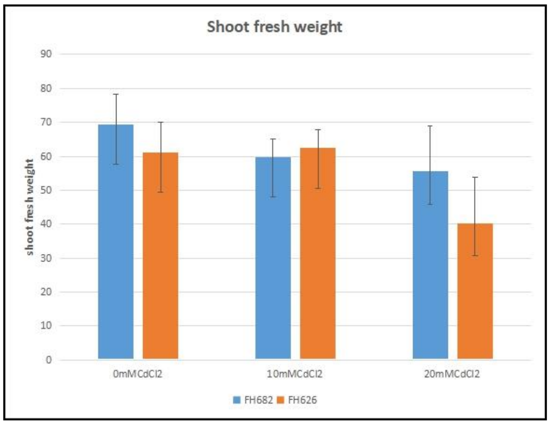

Fig. 3. Shoot fresh weight in FH682, FH626 Treatment (0, 10 and 20) mM.

Analysis of variance of data for root fresh weight of sunflower variety FH682.FH626 were grown under different concentration of cadmium chloride ($CdCL_2$) A significant increase was observed in root fresh weight of control series as compared to 10,20mM treatment. while maximum decrease was observed when 0mM $CdCl_2$ as applied. Variety FH626 showed more root fresh weight as compared to Variety FH682.

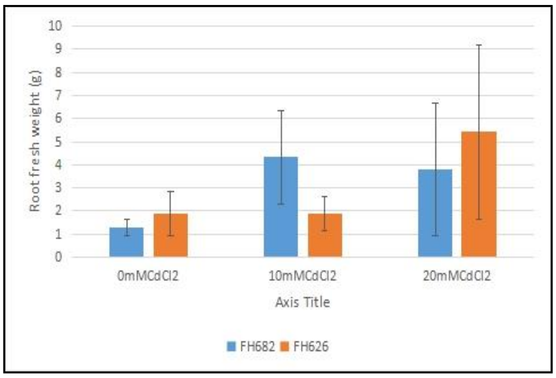

Fig. 4. Root fresh weight in FH682, FH626 Treatment (0, 10, 20) mM.

Examination of variance of data for shoot dry weight of sunflower variety FH682.FH626 were grown under different concentration of cadmium chloride ($CdCL_2$) A significant increase was observed in shoot dry weight of control series as compared to 10,20mM treatment. while maximum decrease was observed when 0mM $CdCl_2$ as applied. Variety V2 showed more shoot dry weight as compared to Variety v1.

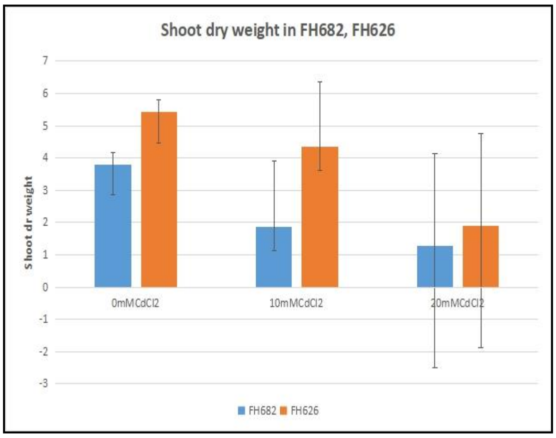

Fig. 5. Shoot dry weight in FH682, FH626 Treatment (0, 10, 20)mM

For the investigation of variance of data for root dry weight of sunflower FH682.FH626 were grown under different concentration of cadmium chloride ($CdCL_2$) A weighty increase was observed in root dry weight. while maximum decrease was observed when 0mM CdCl2 as applied. Sunflower performs better 10mM, 20mM concentration of $CdCl_2$. Variety V2 showed more root dry weight as compared to Variety v1.

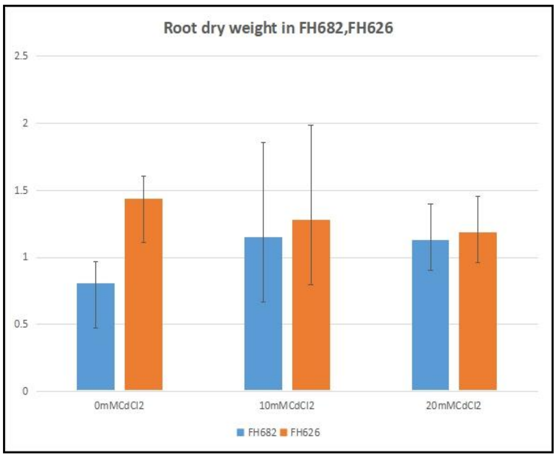

Fig. 6. Root dry weight in FH682,FH626 Treatment (0,10,20)mM.

Analysis of variance of data for chlorophyll a stress application decrease chlorophyll a content in plants some plants die 10 mM concentration cause less damage to chlorophyll degradation as compared to 20mM concentration solution the leaves and shoot have less green colure as compared to control series. Variety V2 showed more chlorophyll a contents as compared to Variety v1.

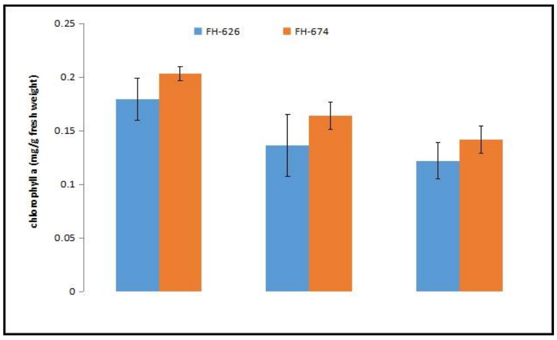

Fig. 7. Pigments system (chlorophyll a, as mg g fresh weight-1) of H. annuus. L FH682, FH626 Treatment (0, 10,20)mM.

Analysis of variance of data for chlorophyll a stress application decrease chlorophyll b content in plants some plants die after stress application 10 mM concentration cause less damage to chlorophyll degradation as compared to 20mM concentration solution. The chlorophyll degradation cause degradation of green colure in plants in stress applied plants. Variety V2 showed more chlorophyll b contents as compared to Variety v1 or show more green colure as compared to treatment.

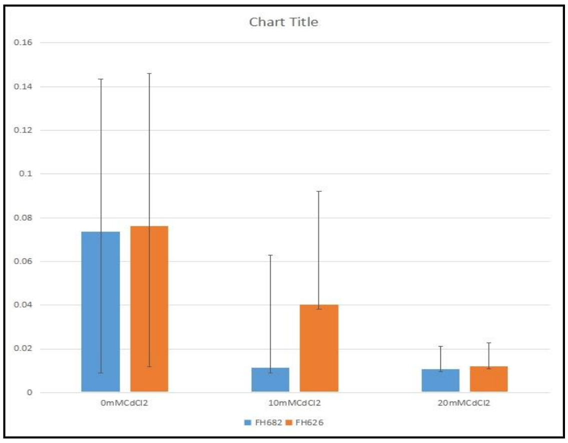

Fig. 8. Pigments system (chlorophyll b, as mg g fresh weight-1) of H. annuus. L FH682, FH626 Treatment (0, 10, 20) mM.

After analysis of variance of data for total chlorophyll a /b stress application decrease total chlorophyll a/b concentration in stress applied plants some plants die after stress application 10 mM concentration cause less damage to chlorophyll degradation as compared to 20mM concentration solution. Variety V2 showed more chlorophyll a/b values as compared to Variety v1.

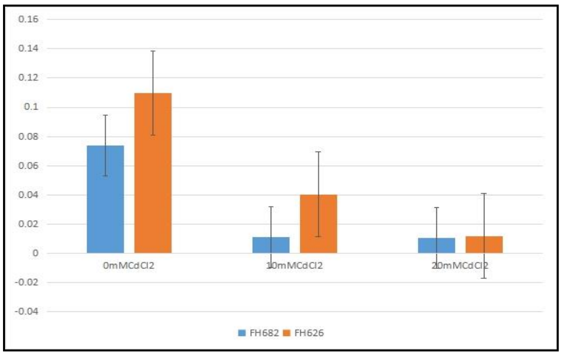

Fig. 9. Pigments system total (chlorophyll a,b, as mg g fresh weight-1) of H. annuus. L FH682, FH626 Treatment (0, 10, 20) mM.

Analysis of variance of data for chlorophyll a stress application decrease carotenoids content in plants some plants die 10 mM concentration cause less damage to chlorophyll degradation as compared to 20mM concentration solution. The reduction in chlorophyll content and carotenoids reduce the photosynthetic efficiency of sunflower plants. Variety V2 showed more carotenoid values as compared to Variety v1.

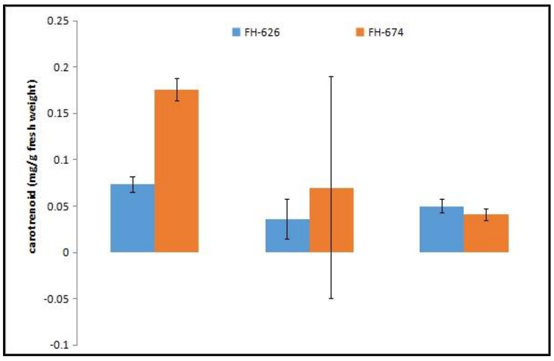

Fig. 10. Pigments system (carotenoids as mg g fresh weight-1) of H. annuus. L FH682, FH626 Treatment (0, 10, 20) mM.

Number of leaves decrease as treatment increase higher in control series than lower both treatments 10,20mM $CdCl_2$.

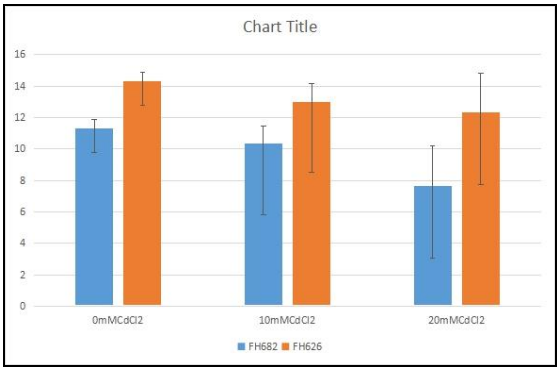

Fig. 11. No of leaves in FH682,FH626 Treatment (0,10,20)mM.

Effect of cadmium on plant development

H. annuus belongs to a select group of plants, being able to develop in soils containing high levels of potentially toxic elements including cadmium. So far, the literature reports little information about the phytotoxic level of cadmium in sunflowers. Thus, the tolerance of sunflowers to the toxic effect of cadmium was investigated at different doses of this element. Among the three groups of plants that received the addition of cadmium during cultivation, 50% of the plants died when considering the group treated with the highest cadmium dose (Cutright *et al.,* 2010).

Cadmium is a non-essential element that usually is taken up by plants due to its high mobility in soil and similar behavior when compared to some essential elements (Arvind *et al.,* 2005). The total concentration of cadmium was determined in different parts of the sunflower plants control and Cd-treated groups to evaluate the strategies used by plants against this exposure. The results obtained for cadmium content in leaves, stems and roots of the sunflower plants. As shown, cadmium uptake by sunflowers was significant and proportional to its spiked concentration during the cultivation period, since the highest cadmium concentration was found in plants irrigated with the highest cadmium dose (Tezotto *et al.,* 2012).

High Cd, moreover, caused a significant reduction in chlorophyll and carotenoid contents in sunflower and in fern. Reduction in chlorophyll content may be associated with the interference of metals with chlorophyll synthesis and lipid metabolism (Pandey and Tripathi, 2011). It can be also related to inhibition of root and shoot growth, photosynthesis, nutrient uptake, leaf area, etc. . Additional K may also play a vital role in the formation of photosynthetic pigments and prevents Chl decompositions. These effects of K in plants could be associated with tolerance of plants to avoid abiotic stresses.

Conclusion

The present work describes some important results focusing on sunflowers and cadmium, as well as their correlations with other elements like copper, iron, magnesium, manganese, phosphorus and zinc, which are important for characterizing some process occurring at the molecular level in this culture. The correlation between enzymes proteins and metals can improve the understanding of some physiological roles regarding sunflower metabolism.

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