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ORIGINAL ARTICLE

Effect of Chromium on Growth, Morphology and Economic Yield of *Helianthus annuus* L. Varieties

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ABSTRACT

This study was conducted to observe the effect of chromium on three varieties of sunflower (Helianthus annuus L.) i.e. PSH-569, PSFH-118 and KBSH-41. A pot culture experiment was performed using sandy soil, clay soil and compost in the ration of 1:1:1. Various doses of heavy metal chromium Cr (VI) ranging from 0, 10, 20, 50, 100 and 200 mg per kilogram of soil mixture were given as aqueous solution of K₂Cr₂O₇ to sunflower plants varieties and the plants were grown in cemented pots for 100 days (maturity). It was reported that with increasing chromium concentration, root length, shoot length number of leaves and number of seeds per plant decreased. At higher concentration of 100 and 200 mg Cr (VI) per kilogram of soil mixture, significant reduction in various morphological parameters including reduction in seed yield was observed in all three varieties of sunflower. Among the three varieties of sunflower, variety PSH-569 show better growth and maximum tolerance to chromium heavy metal in various treatments, where as variety KBSH-41 show least tolerance to chromium growth in chromium treatments among the three varieties of sunflower. **KEY WORDS** :- Helianthus annuus, chromium, phytoremediation, growth, heavy metals.

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INTRODUCTION

Contamination due to pollution of agriculture soil in cultivated land is a major environmental problem and due to rapid urbanization and industrial revolution, this problem has accelerated to an alarming extent, in past few decades. When the heavy metals are present in higher concentration, they may cause serious damage to crop plants [1,2,3]. Reports indicate that heavy metal pollution including chromium in soil arise as a result of various anthropogenic activities, such as continuous use of sewage water in irrigation, sewage sludge [4] and due to indiscriminate use of pesticides and chemical fertilizers in the fields [5]. Heavy metals added via sewage sludge are usually retained in the top 0-15 cm of soil layers and thus are responsible for major reduction in crop yield and contamination of crop [6]. Chromium is found in all phases of environment including air, water, soil [7] and is mainly released from tanneries and pigment industries [8]. Chromium is also received in ecosystem form electroplating industries wood preservation, paper production and sewage sludge [9]. Chromium is toxic to most higher plants at 100 µM kg⁻¹ dry weight but infestation of Mycorrhizal fungi enhances accumulation and tolerance of chromium in H. annuus [10]. Presence of chromium in the external environment leads to changes in growth and development pattern of the plants. Sharma and Iqbal [11] reported that tissue concentration of chromium (52-92) µg Cr g⁻¹ dry weight in five cereal crops, induced adverse effect. Phytoremediation is an integrated, cost effective multidisciplinary approach and emerging green technology for removal of heavy metals from contaminated soils, using hyper accumulating plant species like sunflower (*H. annuus* L.) [12]. Therefore interest has been generated to explore the role of various varieties of sunflower (an important oil yielding crop) in phytoremediation of heavy metal chromium from soil and to explore the effect of this heavy metal on various morphological parameters like root length, shoot length including number of leaves and number of seeds per plant in three sunflower varieties.

MATERIAL AND METHODS

Certified seeds of three varieties of sunflower (*H. annuus* L.) i.e. PSH-569, PSFH-118 and KBSH-41 were collected from Oil Seed Section, Department of Plant Breeding, Chaudhary Charan Singh, Haryana Agriculture University, Hisar. Healthy seeds of uniform size were washed in fresh running water to eliminate dust and dirt and then surface sterilized by treating them with 0.2 per cent (W/v) mercuric chloride solution for two minutes to eliminate parasites. The seeds were thoroughly washed again in distilled water. The germination per centage of seeds of three varieties were checked by keeping 100 seeds of each variety in glass petriplates (20 centimeter diameter) separately, lined with two layers of Whatman No. 1 filter paper and moistened with distilled water. These petriplates were kept in BOD incubator for 8 days in 12 hrs dark/light photoperiod at $25\pm1^{\circ}$ C temperature and germination.

Experiment was carried out in 15 inches x 15 inches size, polythene lined cemented pots in energy park of Guru Jambheshwar University of Science and Technology, Hisar (Haryana). A soil mixture was prepared on plastic sheet containing sandy soil, clay soil and farm yard manure (FYM), in the ration of 1:1:1. The soil mixture was air dried, grind and passed through 2 mm stainless steel sieve and mixed thoroughly again. Various physiochemical parameters of two soils and farm yard manure were determined like, $pH(H_2O)$, organic carbon (%), electrical conductivity (EC), cation exchange capacity (CEC) total nitrogen, total phosphorus, water holding capacity (%), particle density and heavy metal presence etc.

This soil mixture was mixed with appropriate amount of 10 mg, 20 mg, 50 mg, 100 mg and 200 mg kg⁻¹ soil Cr(VI) doses separately, in solution form, by using desired volume of Analytical Reagent (AR) grade K₂Cr₂O₇. After 5 days, the entire contents from the pots were taken out, mixed thoroughly again and refilled in pots @ 8kg soil mixture pot⁻¹ and incubated for another five days. After filling the soil mixture, the pots were wetted with deionised water to maintain appropriate moisture content to nearly 30% and maintained it time to time at workable moisture level. Seeds of three varieties of *H. annuus* L. were washed and wetted with distilled water for 30 minutes and then treated with 0.2% (W/v) mercuric chloride solution for 2 minutes and again washed two times with distilled water and after that five healthy seeds of three varieties were sown in each pot. Thinning was done after the emergence for seedlings and only one plant per pot was kept intact. These pots were irrigated with distilled water, as and when required depending on the water holding capacity of soil, so that no loss of water/minerals took place from pots. The plants were grown for 100 days to attain maturity and flowering. The metal toxicity symptoms and growth parameters such as shoot length, number of leaves per plant and number of seeds per plant were recorded before harvesting and root length was recorded after harvesting the crop at maturity, after completing the age of 100 days. For data analysis, factorial CRD for two factors were used to examine and analyse the effect of heavy metals on three varieties of sunflower. The mean, critical difference (CD), standard deviation (SD) and standard error (SE) were determined statistically.

RESULTS AND DISCUSSION

Table 1-3 show various physiochemical characteristics of sandy soil, sandy clay soil and farm yard manure (FYM) used for filling the cemented pots for sowing the experimental crop varieties. Table 4.0 showed the effect of chromium on root length of three varieties of sunflower. The mean root length in control was 32.39 cm, which reduced to 30.76, 26.48, 23.85, 18.84 and 14.86 cm in 10, 20, 50, 100 and 200 mg kg⁻¹ soil chromium concentration. Results revealed that increasing concentration of chromium decreased the root length in all three varieties of sunflower, but this decrease in mean root length due to effect of chromium was less in lower doses of chromium up to 50 mg kg⁻¹ of soil as compared to decrease in mean root length in higher doses of 80, 100 and 200 mg kg⁻¹ soil, thus high conc. of Cr (VI) is more toxic to root development and root growth than low chromium concentration. The bioaccumulation of heavy metals Cr, Fe, Ni, Pb, Zn and Cu and removal of these heavy metals from the soil using like *Pangamia, Chenopodium, Cassia* and *Moringa* have well documented [13]. Chen et.al. [14], also noted that total root weight and root length in wheat plants was affected by 20 mg Cr (VI) kg⁻¹ soil. From the result of Table 4.0, it is clear that the mean root length was maximum i.e. 25.11 cm in variety PSH-569 was found to exhibit greater tolerance for chromium metals, over the other two varieties.

From the perusal of data in Table 5.0, the effect of chromium on shoot in 100 days old sunflower plants varieties was observed and it was found that with increasing concentration of chromium, shoot length was also decreased gradually in all three varieties of sunflower. The mean value of shoot length was 142.78 cm in control, which decreased to 125.67, 119.84, 108.18, 86.29 and 64.00 cm in 10, 20, 50, 100 and 200 mg kg⁻¹ soil chromium, causing a reduction in shoot growth and shoot biomass. This decrease in shoot length was less at lower doses of chromium i.e. at 10, 20 and 50 mg Cr (VI) kg⁻¹ soil but found more

inhibitory at higher doses of 100 and 200 mg Cr (VI) kg⁻¹ soil chromium level. However the varietal difference of mean root among the three varieties of sunflower was not significant (V = 1.0598). The above results are matching the results obtained by Hanus and Tomas [15], as they observed a significant reduction in plant height in *Sinapis alba* when Cr (VI) was supplied at the rates of 200 or 400 mg kg⁻¹ soil along with N, P, K and S fertilizers. Adverse effect of Cr on plant height and shoot growth was reported by Rout et.al. [16], when Cr (VI) was added at 2 ppm, 10 ppm and 25 ppm to nutrient solutions in sand culture in Oats.

 Table 1:- Physicochemical characteristics of sandy soil, collected from sand dunes of Balsamand village, Bhadra Road, Hisar.

Physicochemical Parameters	Values	
pH*	8.2	
EC (dSm ⁻¹)*	0.36	
CEC [Cmol (P+) kg-1]	3.50	
Total Organic Carbon (%)	0.12	
Total Nitrogen (%)	0.23	
Total Phosphorus (gkg-1)	0.2	
CaCO ₃ (%)	0.73	
Heavy metal contents (mgkg ⁻¹)		
Iron (Fe)	0.45	
Lead (Pb)	<mdl< td=""><td></td></mdl<>	
Cadmium (Cd)	<mdl< td=""><td></td></mdl<>	
Chromium (Cr)	<mdl< td=""><td></td></mdl<>	
Nickel (Ni)	<mdl< td=""><td></td></mdl<>	
Copper (Cu)	0.027	
Texture	Sand	
Water Holding Capacity (%)	32	
Porosity (%)	29.8 %	

*1:2 Soil: Water suspension, MDL values: Pb-0.06, Cd-0.01, Cr-0.05, Ni-0.03mg/I

Table 2:- Physicochemical characteristics of soil collected from Energy Park, GJU of Sci. & Tech., Hisar.

Physicochemical Parameters	Values
pH*	8.4
EC (dSm ⁻¹)*	0.5
CEC (meq./100g of soil)	0.904
Total organic carbon (gkg-1)	31.8
Total Nitrogen (gkg-1)	2.4
Total Phosphorus (gkg-1)	0.8
Water Holding Capacity (%)	62
Bulk Density (Db)	1.33
Particle Density (Dp)	2.398
Porosity (%)	44.4
Textural class	Sandy clay
Heavy metal contents (mgkg-1)	
Cadmium (Cd)	<mdl< td=""></mdl<>
Chromium (Cr)	<mdl< td=""></mdl<>
Copper (Cu)	<mdl< td=""></mdl<>
Nickel (Ni)	0.028
Zinc (Zn)	7.5

*1:2 Soil: Water suspension, MDL values: Cu-0.025, Cd-0.01, Cr-0.05 mg/l.

Physicochemical Parameters	Values				
pH*	7.3				
Electrical conducting (EC)(dS/m)*	1.87				
Total organic carbon (g/kg)	279				
Total K Nitrogen (g/kg)	5.2				
Phosphorus (g/kg)	7.2				
Total Potassium (g/kg)	6.0				
C/N ratio	53.65				
C/P ratio	38.75				
Heavy metal contents (mg/kg-1)					
Cadmium (Cd)	<mdl< td=""><td></td></mdl<>				
Nickle (Ni)	1.61				
Zinc (Zn)	23.5				
Copper (Cu)	21.8				
Chromium (Cr)	<mdl< td=""><td></td></mdl<>				

*1:2 Soil: Water suspension, MDL values: Cd-0.01, Cr-0.05.

Table 4:- Effect of chromium on root length (cm) at maturity in 100 days old plants in Helianthus annuus L.
(Mean±SE)

Varieties	Cr (VI) conc. mg kg ⁻¹ soil						
	0	10	20	50	100	200	MEAN
PSH-569	32.93±0.15	31.56±0.2	26.96±0.45	24.93±0.19	19.03±0.22	15.23±0.32	25.11
PSFH-118	32.30±0.3	29.86±0.33	26.73±0.33	24.36±0.32	19.10±0.31	15.33±0.3	24.61
KBSH-41	31.93±0.52	30.86±0.26	25.73±0.2	22.26±0.64	18.40±0.13	14.03±0.18	23.87
MEAN	32.39	30.76	26.48	23.85	18.84	14.86	

CD (P<0.05), V = 0.398, L = 0.564, V x L = 0.976

 Table 5:- Effect of chromium on shoot length (cm) at maturity in 100 days old plants in Helianthus annuus L.

 (Mean+SE)

Varieties	Cr (VI) conc. mg kg ⁻¹ soil						
	0	10	20	50	100	200	MEAN
PSH-569	146.6±0.71	138.63±0.35	122.8±0.67	108.5±0.64	87.16±0.73	63.4±1.42	111.18
PSFH-118	144.76±0.49	126.8±0.49	126.56±0.52	107.8±0.67	89.37±0.49	74.43±0.5	111.62
KBSH-41	136.97±0.5	111.57±0.41	110.17±0.26	108.23±0.2	82.33±2.74	54.17±0.98	100.57
MEAN	142.78	125.67	119.84	108.18	86.29	64.0	

Table 6:- Effect of chromium on numbers of leaves at maturity in 100 days old plants in Helianthus annuus L.

(Mean±SE)

Varieties	Cr(VI) conc. mg kg ⁻¹ soil						
	0	10	20	50	100	200	MEAN
PSH-569	21.66±0.67	21.66±0.39	18.33±0.33	16.00±0.0	13.66±0.88	8.66±0.33	16.66
PSFH-118	22.33±0.67	20.00±0.33	17.00±0.27	16.66±0.33	12.66±0.33	8.33±0.66	16.16
KBSH-41	22.66±0.33	21.66±0.33	16.66±0.33	15.33±0.33	11.33±0.33	7.00±0.57	15.77
MEAN	22.22	21.11	17.33	16.00	12.55	8.00	
MEAN	22.22	21.11	17.33	16.00	12.55	8.00	

CD (P<0.05), V = 0.560, L = 0.792, $V \times L = 1.372$

Varieties	Cr(VI) conc. mg kg ⁻¹ soil						
	0	10	20	50	100	200	MEAN
PSH-569	290.66±4.410	248.33±4.48	232.00±8.18	191.66±4.97	108.66±13.29	0±0	178.56
PSFH-118	285.66±4.1	242.66±6.17	223.0±3.21	195.0±4.35	93.66±4.05	14.33±7.84	175.22
KBSH-41	250.0±4.36	230.0±4.35	212.33±5.23	173.33±5.17	85.66±6.74	7.0±7.0	159.72
MEAN	275.44	240.33	222.44	186.66	96.0	7.11	
		10.010 1/ 1	17010				

CD (P<0.05), V = 7.080, L = 10.013, V x L = 17.343

Sharma and Srivastava [17] also observed maximum dry matter yield in PSH-569 variety and minimum in KBSH-41 variety of *H. annuus* L. in various doses of Cr (VI) and EDTA + Cr (VI) ranging from 10 to 200 mg kg⁻¹ soil chromium level. Sharma and Sharma [18] reported that plant height reduced significantly in 32 and 96 days old wheat variety (CV. UP, 2003), when wheat were sown in sand with 0.5 μ M sodium dichromate. The inhibition of growth of root and shoot due to toxic effects of chromium might be due to its structural similarity with some essential elements which can effect mineral nutrition uptake, translocation and accumulation of essential minerals for biosynthesis of structural molecules required during early growth and development which culminates in reduction in growth, development and also reduction in yield and total dry matter in three varieties of sunflower.

With the perusal of data from Table 6.0 the effect of chromium on number of leaves in 100 days old plants of three sunflower varieties was revealed. The mean number of leaves were 22.22 at 0 level of chromium, which reduced to 20.44, 18.67, 16.11, 14.67 and 11.67 in 10, 20, 50, 100 and 200 mg kg⁻¹ soil chromium concentration, depicting that higher concentration of chromium has inhibitory effect on leaf number in all three varieties of sunflower. Leaf growth and leaf number decisively determine the yield of crops. The results obtained in above data are matching with the results [18], as they observed a reduction of 50% leaf number per plants in wheat when 0.5 mM Cr (VI) was added in nutrient solution. Tripathi et.al. [19] found that leaf area biomass of *Albezia lebbek* seedling was severely affected by 200 ppm Cr (VI) concentration. In higher chromium concentration of 100 and 200 mg kg⁻¹ soil, along with reduced size of leaf, the chlorosis of leaves and burning of leaf tips and margins and necrosis were also observed in all three varieties sunflower plants, in the above study. Also in a study on the effect of Cr (III) and Cr (VI) on spinach, [20] reported that Cr (VI) applied at 60 mg kg⁻¹ soil and higher levels, reduced the leaf size, caused burning of leaf tips and margins and plants observed slow leaf growth. Jain et.al. [21] observed leaf chlorosis at 40 ppm, that turned to necrosis at 80 ppm Cr (VI).

In all the oil yielding plants seeds are the most useful and economically important part of the plant. Therefore effect of heavy metals on number of seeds per plant is a major concern and important aspect of this study. From the results obtained in table 7.0 it is clear that as the chromium dose increased from 0 to 200 mg per kg soil, number of seeds also reduced to 275.44, 240.33, 222.44, 186.66, 96.0 and 7.11 seeds per plant. The results in Table 7.0 are corresponding with the results obtained by [22], who reported a reduction in the yield of barley and maize in pot experiment in 100 mg kg⁻¹ and 300 mg kg⁻¹ soil chromium level. Sharma and Mehrotra [23] found in *Triticum aestivum* L. cv HD 2204, that the seed dry weight yield was 2.11 g per plant without chromium in control which reduced to 0.39 and 0.16 g with 20 and 200 ppm Cr respectively. This decrease in seed yield and reduction in number of seeds per plant may be due to the presence of heavy metal Cr (VI) in the medium which influences cellular structure, reduced rate of cell division during embryonic development and also due to reduced translocation of nutrient minerals, reserved proteins and carbohydrates required during seed growth and development.

The result obtained from Table 4.0 to 7.0 on morphological parameter and economic yield indicate that among all three sunflower varieties, the PSH-569 variety show better growth and tolerance to the heavy metal chromium in comparison to other two varieties, where as KBSH-41 variety is least adapted to metal tolerance and show its minimum vegetative growth and minimum seed yield. Thus the variety PSH-569 may be a better recommended variety for phytoremediation practice, among the three varieties of sunflower up to 20 mg Cr (VI) per kilogram of soil.

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