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Full Length Research Paper

Effect of tannery effluents on seed germination and growth of two sunflower cultivars

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The wastewater of a tannery in Multan, Pakistan, was alkaline with high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) values along with much higher concentrations of total settle able salts and suspended solids, sodium adsorption ratio and high amount of sodium having the water quality class C_3S_1. Effluent was examined for its chemical constituents and the effect of its various dilutions was examined in greenhouse on two newly recommended sunflower cultivars (FH-330 and FH-245) during their whole growth period. Percentage of germination, chlorophyll, carbohydrates and protein contents of both the sunflower cultivars showed significant (p = 0.05) decreasing trend with increasing effluent concentrations. Vegetative growth parameters like plant height and number of leaves per plant were significantly (p = 0.05) reduced with the increasing levels of effluents. Rate of leaf senescence of both cultivars was higher under higher effluent concentrations. Yield of sunflower crop in both cultivars was significantly (p = 0.05) reduced due to effluent concentrations. Seeds per capitulum were decreased to 49%, seeds weight per plant to 61 - 66% and 100-seed weight to 49 - 59%. The appearance of pale yellowish color of the affected plants was due to reduction in photosynthetic material under higher effluent concentration. Full strength effluent concentration caused the reduction in biomass accumulation and reproductive growth of sunflower cultivars. The results revealed that cultivar FH-330 was relatively resistant to varying effluent concentrations as compared to the cultivar FH- 245. However, the tannery effluents due to the presence of chemicals are not suitable for inclusion in irrigation system.

Key words: Tannery effluents, sunflower, seed germination, growth, adverse effect, reduced yield.

INTRODUCTION

Human civilization originated, developed and thrived in places within easy reach of fresh water sources. Amongst global resources, water is emerging as perhaps the most critical but misused natural resource (Saleemi, 1993). Water is the elixir of life without which no biota could survive in the biosphere. The industrial growth in Pakistan though solving economic problem, also add up to environmental pollution as waste water effluents from tanneries are discharged into water bodies deteriorating the water quality (Faisal and Husnain, 2004; Qureshi and Barrett-Lennard, 1998). The discharge of industrial effluents, besides increasing the dissolved residues which increase the total amount of sediments, also bring about chemical transformation in soils continuously irrigated by polluted waters (Faisal and Husnain, 2004). As a result of this, serious biological and ecological instability may occur as more and more industrial wastes are thrown out into the bodies of water. At higher pollution levels, root system is extremely lost and at maturity, plants yield are much reduced (Joshi et al., 1999; Mondal et al., 2005; Yasir, 2003). Waste water from industries also destroys our productive land by adding chemical compounds to these soils. Delayed germination and earlier leaf senescence are the two most important parameters which correspond to the final yield loss at the end of the season (Bishoni, 1993; Clemente et al., 2005). Soluble minerals in polluted water actually

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Abbreviations: EC, Electrical conductivity; **SAR,** sodium absorption ratio; **RSC,** residual sodium carbonate; **SS,** suspended solids; **BOD,** biochemical oxygen demand; **COD,** chemical oxygen demand; **TCA,** trichloroacetic acid.

enhance the salinity problems close to several industries (Davidson, 2000). World society is faced with the challenge of controlling the environmental problems and to enhance crop production under threat of drastic environmental conditions. There should be a concerted effort to entirely reduce or avoid environmental pollution. This would help solve the ecological crisis and will provide clean environment for unrestricted explosion of human population.

Sunflower (*Helianthus annus* L.) is ranked fourth in the world among source of vegetable oils. Sunflower seed oil has a production of about 18% of the world production edible vegetable oils. For human consumption, sunflower oil is equal to the finest olive oil for its food value, lack of taste, color and keeping qualities. It is probably in the top rank for salad oil, cooking, frying, canning and medicinal purposes. Present study has been envisaged to study the effect of tanneries wastewater effluents on seed germination and growth performance of sunflower cultivars FH-330 and FH-245 as it is an important crop of the region.

MATERIALS AND METHODS

Effluent samples of a tannery in Multan, Pakistan were collected from the main outfall in 30 liter plastic cans. The name of the tannery is deliberately omitted to protect the privacy of the industry. Industrial effluents were analyzed for physicochemical characteristics following Rainwater and Thatcher (1960), APHA (1976) and RUMPH and Krist (1988) through the courtesy of Environmental Protection Department, (EPD) Government of Punjab, Lahore. The wastewater was light grey in color and contained a number of solid waste materials such as hair, pieces of skin and flesh.

Analysis of wastewater effluent

Effluent sample was analyzed for its physiochemical characteristics. Calcium was estimated with the help of flame photometer. Estimation of Mg^+, Cr_3^{++} and Zn^+ was made by atomic absorption spectrophotometer. CO_3 and HCO_3 were estimated by titration method. The values of electrical conductivity (EC), sodium absorption ratio (SAR), residual sodium carbonate (RSC), suspended solids (SS), biochemical oxygen demand (BOD) and chemical oxygen demand (COD) were also determined. Water quality class was determined following Richards (1954). Cl^- was estimated by silver nitrate titration method and the remaining determinations were made following the methods of Richards (1954).

Germination experiment

Six test solutions (0, 20, 40, 60, 80 and 100% v/v) prepared by diluting tannery effluent with distilled water were used to investigate the effects of wastewater on germination of two cultivars of sunflower (FH-330 and FH-245). Ten healthy and undamaged seeds of equal size were evenly placed in each sterilized Petri dish which contained water soaked filter papers. The Petri dishes were arranged in completely randomized block design. Measured quantities of test solutions was added in each replicate and exposed for 8 h. Seedlings were then grown in distilled water. Random samples were taken from each treatment after 7 days and chlorophyll content was estimated using the flag leaf. The absorbance was taken using UV-spectrophotometer. The chlorophyll a and b were determined following the method of Arnon (1949). The trichloroacetic acid (TCA) insoluble protein of shoot and root was measured by the procedure described by Lowry et al. (1951), using bovine serum albumin as standard. Carbohydrate contents of shoot and root were measured according to the method of Yemm and Willis (1954). Germination potential, seedling length, fresh and oven dry weight were also determined.

Growth experiment

The growth experiment was carried out in a wire netting house at Botanical Garden, Bahauddin Zakariya University Multan, Pakistan, in a good environment and proper season spreading from February to June, 2005. This study was conducted to determine the effect of tannery effluents on growth performance and yield components of two cultivar of sunflower. Experimental set up consisted of ten replicate pots per treatment and there were total of six treatments made by diluting tannery effluent (v/v) with distilled water (T0 = 0%, T1 = 20%, T2 = 40%, T3 = 60%, T4 = 80% and T5 = 100%). T0 served as control. Experiment was arranged in completely randomized design. The pots were filled with field soil and cow manure in the ratio of 6: 2. There were two plants per replicate pot constituting 240 plants of both the cultivars for growth data collection. Plant growth and development were recorded throughout the growing season through biweekly measurements. Two main destructive harvests were made. The first harvest designated as mid-season harvest consisted of 5 pots per treatment and was taken when plants had completed their vegetative growth. The second harvest was taken at the approach of crop maturity. This harvest also included 5 pots per treatment.

Statistical analysis of data

Treatments means, standard error, least significant difference (LSD) and Duncan's Multiple Range Test (Steel and Torrie, 1960) were calculated from the data obtained for various parameters of growth and yield experiments using software package Costat version 3.03.

RESULTS

Quality of waste water effluents

The results of chemical analysis of waste water effluent of a tannery are presented in Table 1. The pH value of full strength (100%) effluent water was highly basic. BOD and COD were very high showing that no life sign could be present in the effluents. Effluent was very rich in Ca^{+2}, Mg, Na, Cl and suspended solids. Hardiness was also very strong rendering it unfit for domestic use. As the amount of HCO_3 was less than the calcium and magnesium, the water had no residual sodium carbonate. For irrigation purposes, water is usually classified for its quality on the basis of electrical conductivity and sodium adsorption criteria. According to this classification, full strength waste water was very high in salts and placed in C_3-S_1 category that is, highly saline and low sodium water.

Table 1. Analysis of wastewater effluents from a tannery in Multan, Pakistan.

Parameters	Tannery constituents
Color	Light grey
Temperature	34°C
pH	8.6
Electrical conductivity (EC)	1490 m Siem/cm
Total settle-able solids	690 meq/l
Total dissolved solids	3688 meq/l
Total suspended solids	987 meq/l
Biochemical oxygen demand (BOD)	680 meq/l
Chemical oxygen demand (COD)	1600 meq/l
Hardness	260 ppm
Calcium + Magnesium	60 meq/l
Sodium	107 meq/l
Chromium	3.7 meq/l
Zinc	-
Lead	-
Carbonate	-
Bicarbonate	4.32 meq/l
Chloride	820 meq/l
Sulphate	1707 meq/l
Total cations	9.31 meq/l
Total anions	7.65 meq/l
Copper	-
Sodium adsorption ratio (SAR)	8.65
Residual sodium carbonate	4.5
Water quality class	C_3S_1

Percentage germination

Percentage germination was initially delayed with increasing levels of effluent water but it became relatively faster after 4 days. Maximum plumules emergence occurred in T0,T1 and T2 treatments, 5 days after sowing and gradually declined significantly ($p = 0.05$) in T3 and T4 treatments. Severe delay in plumules emergence was recorded in T5 starting from day 3 to 7 day. However, after 8 days, 100% germination had occurred in all the six treatment (Table 2).

Growth of seedlings

Fresh and dry weight and length of 15 days old seedling of both the cultivars decreased significantly ($p = 0.05$) with increasing effluent concentrations (T4 and T5). The chlorophyll contents, carbohydrates and proteins were much higher in control followed by other treatments. There was not much difference in the early growth of seedling except that seedlings of T1 treatment emerged a day earlier than those from T4 and T5 treatments. But in the case of T4 and T5 treatments, seedling emergence was slightly delayed. The plants from T1 treatment were lush green and healthier than the plants in T2, T3, T4 and T5 treatments.

Biomass assay

Data regarding various parameters of biomass are presented in Figures 2 and 3. The first harvest was taken after 9 weeks when the plants were at the prime of vegetative growth. There was a significant ($p = 0.05$) percent reduction in plant fresh weight, plant dry weight, plant length, leaf area and chlorophyll contents in different effluent concentrations as compared to control. It may be noted from the results that shoot and root system in plants from control and T1 treatments was better developed as compared to other treatments. The plants from control and T1 treatment were much healthier with maximum number of leaves and branches and were heavier in weight. The plants in T4 and T5 treatments were less developed and lighter in weight. The amount of chlorophyll (total as well as chlorophyll ‘’a’’ and chlorophyll “b”) was highest in plants of T0 and T1 treatments followed by successive and statistically significant ($p = 0.05$) gradual reduction in plants supplied with increasing effluent level. It may also be noted that the amount of

Table 2. Effect of different concentrations of tannery effluents on the germination potential of two sunflower cultivars.

Sunflower cultivars	Treatments	Germination (%)						
		2 days*	3 days*	4 days*	5 days*	6 days*	7 days*	8 days*
FH-330	T0 (Cont.)	42.57a±0.71	64.79a±1.72	95.32a±1.89	100a±0.00	100a±0.00	100a±0.00	100a±0.00
	T1 (20%)	35.24b±0.73	56.35b±1.68	90.35b±1.64	100a±0.00	100a±0.00	100a±0.00	100a±0.00
	T2 (40%)	29.45c±0.90	46.59c±1.63	77.12c±1.33	94.35b±1.63	100a±0.00	100a±0.00	100a±0.00
	T3 (60%)	0.00	35.32d±1.25	64.23d±2.08	87.20c±0.86	95.87b±0.37	100a±0.00	100a±0.00
	T4 (80%)	0.00	26.98e±1.32	38.87e±1.40	56.14d±1.75	74.70c±0.74	95.58b±0.41	100a±0.00
	T5 (100%)	0.00	15.65f±1.49	21.99f±1.97	44.23e±1.22	66.52d±0.89	86.70c±1.03	100a±0.00
FH-245	T0 (Cont.)	39.26a±0.67	59.83a±1.54	92.53a±1.89	100a±0.00	100a±0.00	100a±0.00	100a±0.00
	T1 (20%)	32.32b±0.55	51.23b±1.11	73.25b±1.97	91.90b±1.97	100a±0.00	100a±0.00	100a±0.00
	T2 (40%)	21.19c±0.53	30.18c±1.39	65.19c±1.30	83.43c±0.75	100a±0.00	100a±0.00	100a±0.00
	T3 (60%)	0.00	21.15d±1.55	48.38d±1.17	69.69d±1.78	91.19b±0.40	100a±0.00	100a±0.00
	T4 (80%)	0.00	17.65e±1.49	28.81e±1.66	54.32e±2.19	69.80c±1.35	90.67b±0.60	100a±0.00
	T5 (100%)	0.00	11.43f±1.73	20.93f±1.45	40.29f±1.29	59.31d±1.05	81.68c±0.44	100a±0.00

Mean followed by different letters in the same column are significantly different at p=0.05 according to Duncan's new multiple range test. ± Standard error *: days after sowing.

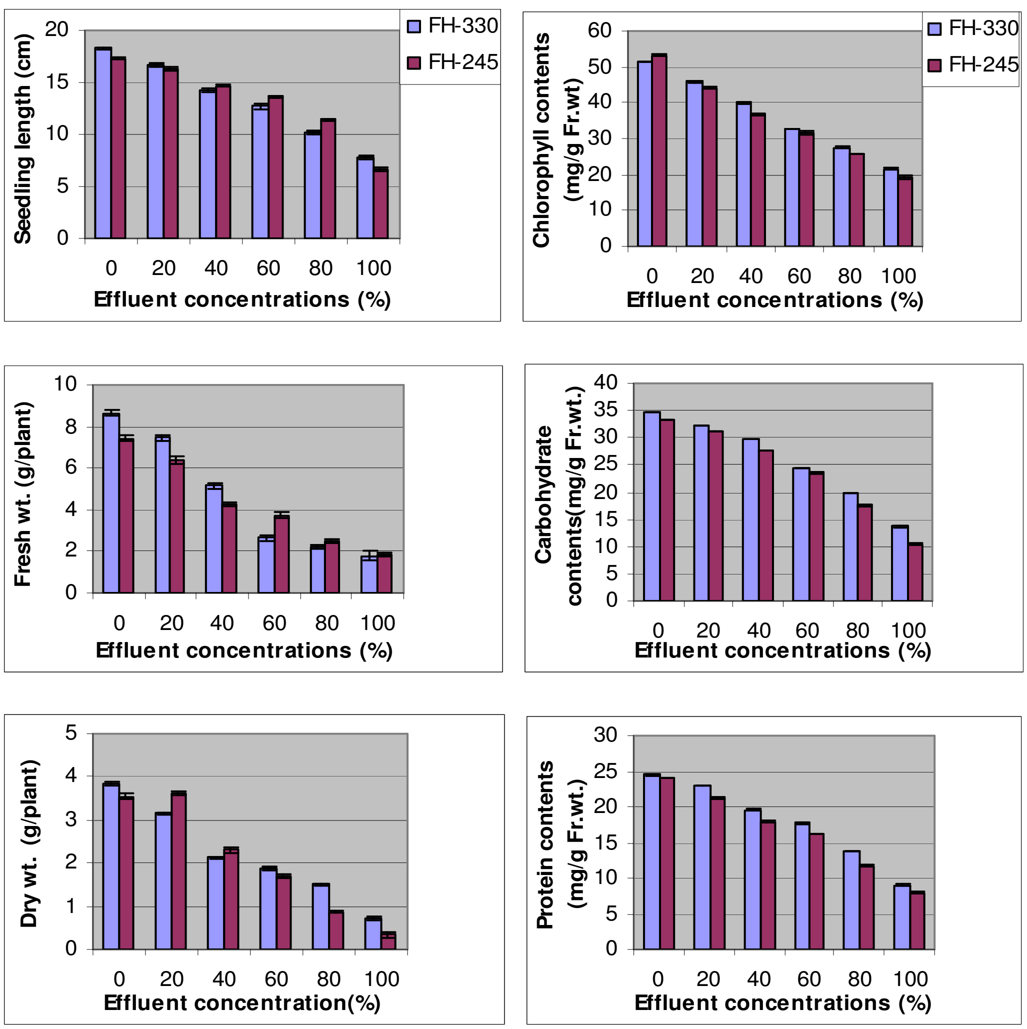

Figure 1. Effect of different concentrations of tannery effluents on the growth and carbohydrate, chlorophyll and protein contents of 15-days old seedlings of sunflower cultivar (FH-330 and FH-245).

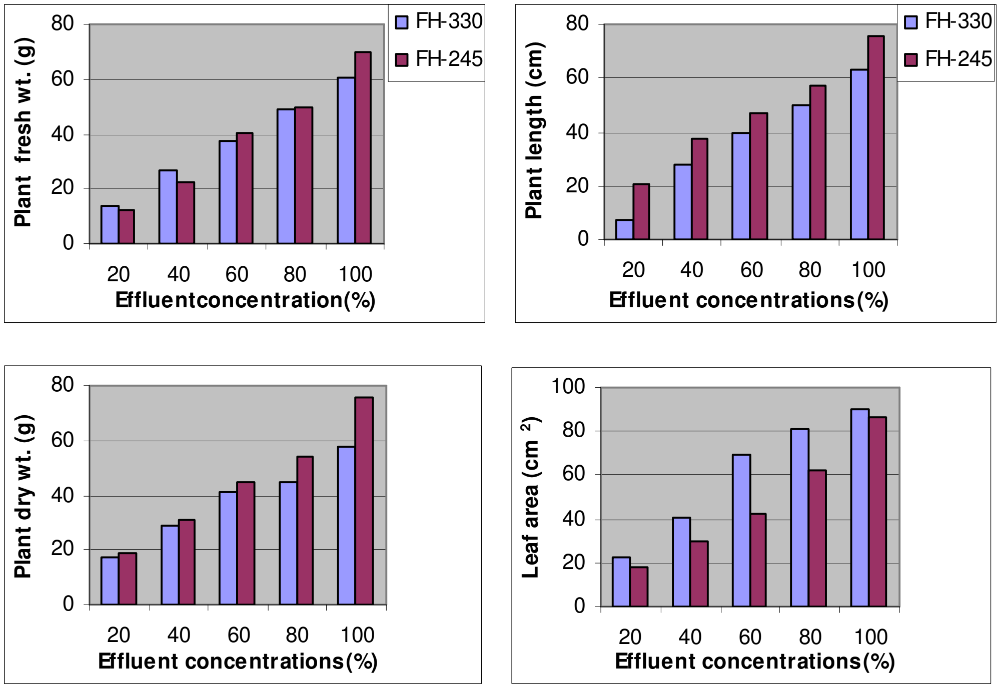

Figure 2. Percentage reduction in growth of 9 weeks old sunflower cultivars FH-330 and FH-245 plants grown under different concentrations of tannery effluents.

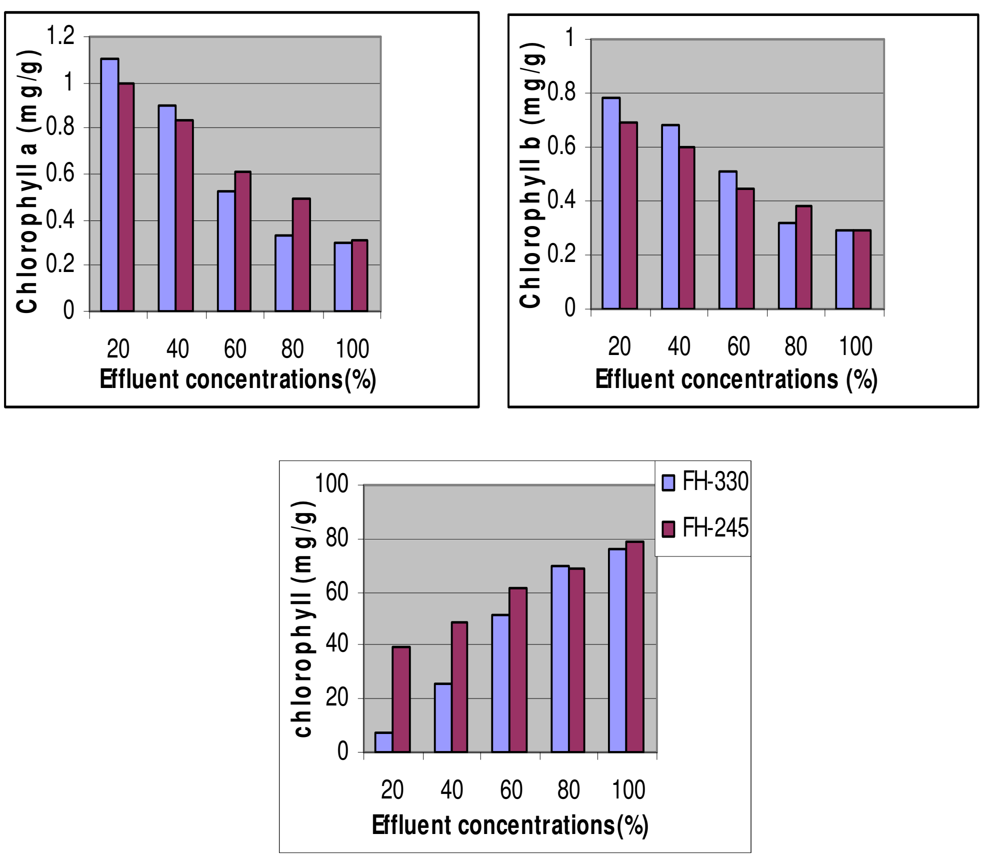

Figure 3. Percentage reduction in chlorophyll contents of 9 weeks old sunflower cultivars FH-330 and FH-245 plants grown under different concentrations of tannery effluents.

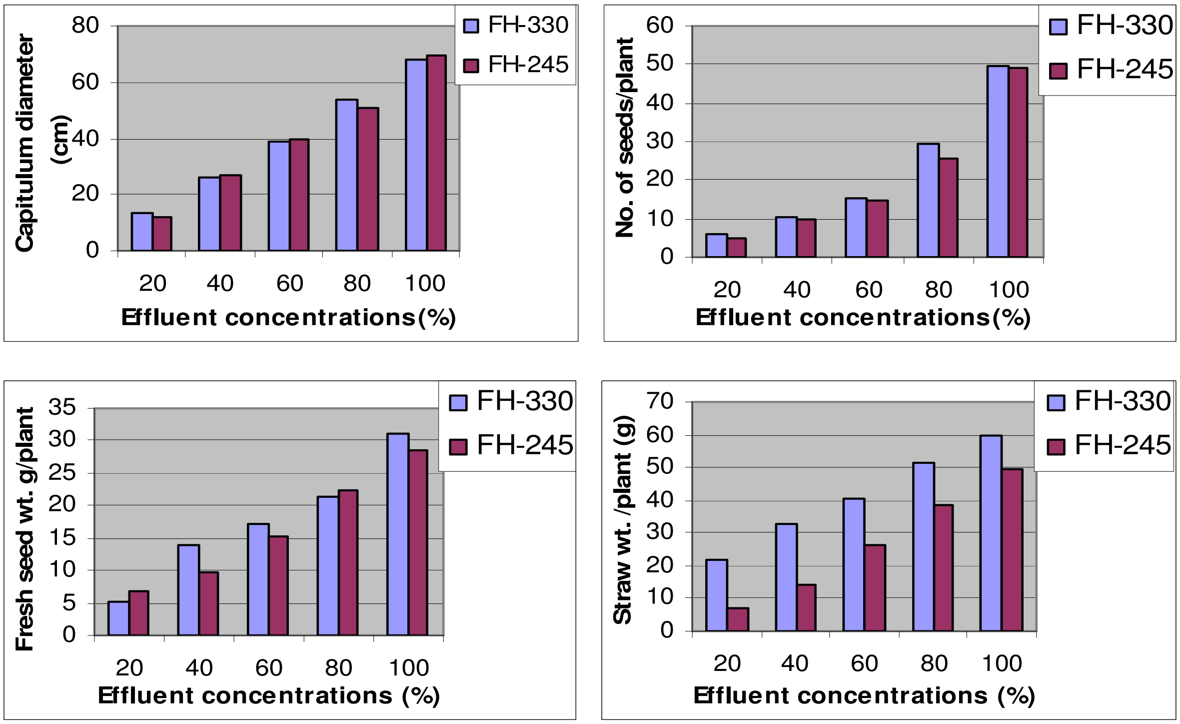

Figure 4. Percentage reduction in yield parameters of 17 weeks old sunflower cultivars FH-330 and FH-245 plants under different concentrations of tannery effluents.

chlorophyll “b” was less than chlorophyll “a” in plants of all the six treatments.

Reproductive growth

Final destructive harvest was taken at crop maturity, 6 to 17 weeks after germination. Plants at this stage were completely dried up with golden yellow color of their straw and capitulm. Various aspects of seed yield as shown in Figures 4 and 5 indicate that capitulum diameter, number of seeds/plant, seed weight per plant, 100 seed weight and straw weight/plant were significantly ($p = 0.05$) reduced with increasing effluent concentrations. Both cultivars (FH-330 and FH-245) of sunflower responded to various effluent levels in a similar pattern. The plant growth was significantly ($p = 0.05$) higher in control and gradually decreased with successive increase in effluent level.

DISCUSSION

Wastewater was analyzed for the assessment of various parameters. The water quality class was highly saline and low sodium (C_3S_1). Effluent contained much higher amount of total dissolved solids, suspended solids, BOD, COD, calcium + magnesium, sodium, chlorides, electrical conductivity, sodium adsorption ratio, pH, total cations and anions as compared to control. The results of effluent analysis at the source are in conformity with the studies of several workers (Algur et al., 1995; Chatterjee and Chatterjee, 2000; Sillen and Martell, 1989; Wahid et al., 1999, 2000). Nearly, all irrigation waters have been used successively for considerable times having electrical conductivity less than 2250 µmhos/cm (Rhoades and Bernstein, 1971). It has been reported that higher chemical concentration has strong inhibitory effects on seed germination (Bishoni, 1993; Jamal et al., 2006a, b, c, d; Yasir, 2003). In the present study, germination was significantly reduced in higher effluent treatments but in lower treatments germination was although affected but not completely suppressed. Similar observations have been made by Mahmood et al. (2005) for seed germination in corn, Jamal et al. (2006a, b, c, d) for seed germination in wheat, *Vigna* spp and *Prosopis juliflora* and Shafiq et al. (2008) for seed germination of *P. juliflora.*

The growth response of both the cultivars to varying effluent concentrations was almost the same but the FH-330 was a little more resistant cultivar. The effect of five concentrations of effluents, on some biochemical analysis of sunflower, both in concentrations and time dimensions, retarded growth of sunflower seedling. All the concentrations of tannery effluent showed an adverse effect on

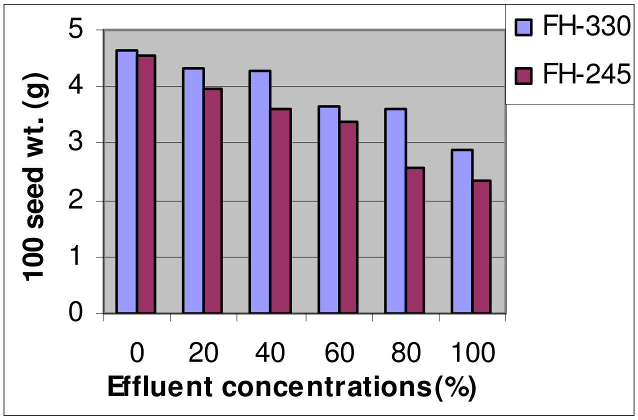

Figure 5. 100 seed weight of 17 weeks old sunflower cultivars FH-330 and FH-245 plants under different concentrations of tannery effluents.

chlorophyll, protein and carbohydrate contents. A gradual decline in the protein and carbohydrate contents with time of exposure to effluents and with concentration of effluent seems to be correlated with the loss of chlorophyll (Gibert and Dubey, 2003). The adverse effects may be due to the higher concentrations of suspended solids together with 18 different types of dissolved chemicals, as reported by Nesmann et al. (1980). Tannery effluents reduced the photosynthetic material in the plants during their early growth and development. The affected plants seem to be pale yellowish in color as compared to the control plants having lush green appearance. The reduction in photosynthetic material in plants grown in higher effluent concentrations showed lesser productivity than the plants grown in less polluted environment (Camplin, 2001; Gren and Trett, 2000) (Figure 1).

Full strength effluent water and their various dilutions caused reduction in biomass accumulation and reproductive growth of sunflower cultivars. In this study, a high decrease was recorded for number of seeds/plant, seed weight/ plant, 100 seed weight and plant dry weight. More than 70% seed yield reduction in higher doses was alarming. But other concentrations of effluents also proved detrimental to plant growth causing 50% reduction in growth and yield. Davies and Linsey (2001) reported grain yield reduction of 69 and 78% in two wheat cultivars due to effluent irrigation. In another study, Turner (2002) reported that effluent caused severe growth reduction in crops, particularly in cereals with marked depression in biomass (50 - 70%).

Conclusion

The water quality class of effluent was highly saline and low sodium (C_3S_1). The results of this study clearly demonstrate that germination was significantly reduced in higher effluent treatment. Biochemical analysis of the cultivars showed both in concentration and time dimensions, retarded growth of sunflower seedling. Effluents application showed adverse effect on chlorophyll, protein and carbohydrate contents. The appearance of pale yellowish color of the affected plants was due to reduction in photosynthetic material under higher effluent concentration. Full strength effluent concentration caused reduction in biomass accumulation and reproductive growth of sunflower cultivars. More than 70% reduction in seed yield was observed under higher effluent dose. The growth response of both the sunflower cultivars was similar but the cultivar FH-330 was more resistant than cultivar FH-245.

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