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Effects of Treated Sugar Mill Effluent Irrigation on Soil and Hybrid Cultivar of Eggplant (*Solanum melongena* L.) under Field Conditions

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Abstract

The disposal of sugar mill effluent is a major problem in India due to generation of huge quantity of effluent in the sugar mills. The present investigation was conducted to study the effects of sugar mill effluent as an alternative of synthetic fertilizers. Six plots were selected for six treatments of sugar mill effluent viz. 0% (control), 20%, 40%, 60%, 80% and 100% for irrigation of *Solanum melongena* L., cv. Pusa Purple Long Hybrid-F_1. The *S. melongena* was grown in sugar mill effluent irrigated soil till harvest and effects of sugar mill effluent on the soil and agronomical characteristics of *S. melongena* were analyzed. The concentrations of sugar mill effluent produced significant (P<0.05/P<0.01) changes in EC, pH, OC, Na^+, K^+, Ca^{2+}, Mg^{2+}, TKN, PO_4^{3-}, SO_4^{2-}, Cd, Cr, Cu, Fe, Mn and Zn of the soil in both seasons. The agronomic performance of *S. melongena* was increased from 20 to 60% concentrations of the sugar mill effluent in both the seasons compared to controls. The accumulation of heavy metals was increased in the soil and *S. melongena* from 20 to 100% concentrations of the sugar mill effluent in both seasons. The biochemical components like crude proteins, dietary fiber, total carbohydrates and total sugar in *S. melongena* were found maximum with 60% concentration of the sugar mill effluent in both seasons. The contamination factor (Cf) of various metals were in the order of Zn>Mn>Cd>Cu>Fe>Cr for the soil and Fe>Mn>Cu>Zn>Cd>Cr for *S. melongena* in both seasons after irrigation with sugar mill effluent. Thus, sugar mill effluent irrigation increased nutrients in the soil and affected the growth of *S. melongena* in both seasons. Consequently, it can be used for irrigation in the lower proportion (up to 60%) to improve the yield of *S. melongena*.

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Introduction

India is being one of the largest producers of sugar in the world and presently has nearly 650 sugar mills that produce about 15 million tons of sugar and 13 million tons of molasses (spent wash) per year[1-3]. The sugar mill effluent is mainly discharged from floor, waste water and condensate water formed by leakage. The disposal of polluted waste water is one of the main problems of today to be faced in the future with its increased adverse effects[4-7]. Most of the sugar mills are discharging their effluent into the environment without any treatment. It has also been reported that sugar mill effluent contains a high magnitude of pollution load and caused adverse effects on soil and biological system. The effluent constitute a number of physico-chemical elements of suspended and dissolved solids with the high amount of biological oxygen demand (BOD), chemical oxygen demand (COD), chlorides, sulphate, nitrates, calcium, magnesium and metals[8-10].

In addition to that, some traceable amount of heavy metals such as zinc, copper and lead are usually present in the sugar mill effluent. The presence of these chemicals in large quantities in the effluent not only affects plant growth but also collapses the soil properties when used for irrigation[3,5,8]. Therefore, the effluent can be applied to productive uses since it contains nutrients that have the potential for use in agriculture. In agriculture, irrigation water can affect soil characteristics, and agricultural crop growth. Besides this use of effluent reduces fertilizer, and irrigation water cost as it is available without paying any cost and rich in various plant nutrients[5,7,9]. Wastewater irrigation is known to contribute significantly to the heavy metal content of the soils. The heavy metals may also accumulate in the soil at toxic levels as a result of long-term application of untreated wastewaters. They are considered as soil contaminants due to their widespread occurrence, acute and chronic toxicity[10,12].

After long-term application of untreated wastewaters, significant amounts of heavy metals can accumulate in the soil at toxic levels. At present, heavy metals, such as cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), lead (Pb), zinc (Zn), etc., are commonly found in subsurface soil irrigated with wastewater[13,14]. Once the adsorption sites of the soil for heavy metals are saturated, more heavy metals would be distributed in the aqueous phase and the bioavailability of heavy metals would subsequently be enhanced[15-18]. The accumulation of heavy metals in agricultural soils has been a wide concern of the public as well as governmental agencies, due to the food safety issues and potential health risks as well as its detrimental effects on soil ecosystems. In addition, heavy metals pollution has frequently been reported in many contaminated sites[19-21].

Agriculture sector in India has been considered for the major user of water. In the changing scenario, reuse of domestic and industrial wastewater in agriculture for irrigating crops appears to be a beneficial option. Besides being source of irrigation water, these wastewaters contain appreciable amounts of plant nutrients[22-25]. The use of industrial effluents for irrigating agricultural lands is on the rise especially in the peri-urban area. These wastewaters carry appreciable amounts of trace toxic metals[10,18,22]. Although the concentrations of heavy metals in the effluents are low, long-term use of these wastewaters on agricultural lands often results in the build-up of the elevated levels of these metals in soils and crops[14,20,23].

Additionally, industrial effluent is an important source of irrigation water and plant nutrients such as nitrogen N, P, K and trace elements like Na, Ca and Mg. Effluent irrigation can eradicate water shortage; reduce the need for chemical fertilizer and enhance the soil fertility[24-28]. However, unregulated irrigation with untreated effluent poses serious public health risks, as effluent is a major source of heavy metals that cause accumulation in plant parts. The effluent contains heavy metals such as Cd, Cr, nickel (Ni) Cu, Fe and Zn which accumulates in plant and vegetable parts, and cause adverse health effects[16,29-31].

Eggplant (*Solanum melongena* L.) is identified as one of the most valuable veggies packed with essential nutrients[31,32]. The eggplant is a delicate, tropical perennial often cultivated as a tender or half-hardy annual in temperate climates[33]. It is being widely cultivated throughout the world in tropical and subtropical climates. Nutritionally, eggplant is low in fat, protein, and carbohydrates. It is rich in dietary fiber, sugar, sodium and potassium[34]. It also contains important vitamins like A, B6, C, D and calcium, iron and magnesium. Eggplant is used in the cuisine of many countries[33-35]. It is widely used in its native Indian cuisine, like vegetable, chutney, curry, and pickle. Eggplant, due to its texture and bulk, can be used as a meat substitute in vegan and vegetarian cuisine. The juice of eggplant significantly reduces weight, plasma cholesterol levels, and aortic cholesterol content[32,35].

Recently most studies were conducted on few agronomic stages with limited parameters in various crops, but there are few reports on comprehensive agronomic studies at various agronomic stages of these plants[36-42]. Use of industrial effluents on cultivation of *S. melongena* is receiving attention but additional information is required on how this crop responds to various concentrations of different types of effluents[43-47]. Keeping in view the significance of sugar mill effluent in the present scenario of agriculture and availability of nutrients, this investigation was conducted to study the effects of sugar mill effluent on the agronomical characteristics of eggplant (*Solanum melongena* L.) under field conditions.

Materials and Methods

Experimental design

The field trials were conducted in the Experimental Garden of the Department of Zoology and Environmental Sciences, Gurukula Kangri University Haridwar, India (29°55'10.81" N and 78°07'08.12" E), to study the agronomical performance of *S. melongena* grown in sugar mill effluent irrigated soil. The crops were cultivated in the rainy and summer seasons during the year 2012 and 2013. For the cultivation of *S. melongena*, six plots (each plot had an area of 9× 9m^2) were selected for the six treatments of sugar mill effluent *viz.*, 0% (Bore well water), 20% (20% sugar mill effluent + 80% bore well water), 40% (40% sugar mill effluent + 60% bore well water), 60% (60% sugar mill effluent + 40% bore well water), 80% (80% sugar mill effluent + 20% bore well water) and 100% (100% sugar mill effluent). All the six treatments were placed within each of the six plots in a randomized complete block design.

Collection of sugar mill effluent and analysis

The sugar mill effluent samples were collected from effluent treatment plant of the R.B.N.S. Sugar Mill, Laksar, Haridwar (29°44'46"N 78°1'46"E). The sugar mill effluent was collected in the plastic containers from effluent treatment plant located in the campus of the sugar mill. It was brought to the laboratory and analyzed for total dissolved solids (TDS), pH, electrical conductivity (EC), Na^+, K^+, Ca^{2+}, Mg^{2+}, total Kjeldahl nitrogen (TKN), phosphate (PO_4^{3-}), sulphate (SO_4^{2-}), Cd, Cr, Cu, Fe, Mn, Zn, standard plate count (SPC) and most probable number (MPN) following standard methods[49,50] and used for irrigation of *S. melongena*.

Preparation of nursery of *S. melongena*

Seeds of a high yield variety of *S. melongena*, cv. Pusa Purple Long Hybrid-F$_1$, were procured from Indian Council of Agriculture Research (ICAR), Pusa, New Delhi, and sterilized with 0.01% Thiram. The nursery was prepared before one month of transplantation of *S. melongena* in each season. For the nursery preparation 0.6 g seeds of eggplant were sown in the six plots in the rows 5 cm apart on 6-12 mm raised nursery beds (farm yard manure mixed soil). The nursery bed was covered with plastic or straw mulch till seeds germinate[51,52]. The plants were irrigated with sugar mill effluent concentrations as per requirement and other agronomical practices like weeding and hoeing were performed till the plants were transplanted in the field.

Transplantation and cultivation practices of *S. melongena*

Four weeks old plants of *S. melongena* were planted at the end of July 2012 and 2013 for the rainy season crop and at the end of April 2012 and 2013 for the summer season crop. Plants of *S. melongena* were transplanted in 6 rows with a distance of 60×60 cm between plants[51,52]. The plants in each plot were irrigated twice in a month with 100 gallons of sugar mill effluent concentrations along with bore well water (control) and necessary agronomical practices were performed till the harvest. The soil was analyzed prior to planting and after harvest for various physico-chemical parameters like soil texture, bulk density (BD), water holding capacity (WHC), EC, pH, OC, Na^+, K^+, Ca^{2+}, Mg^{2+}, PO_4^{3-}, SO_4^{2-}, TKN, Cd, Cr, Cu, Fe, Mn and Zn determined following standard methods[50].

Study of crop parameters

The agronomic parameters of *S. melongena* at different stages (0-110 days) were determined following standard methods for seed germination, plant height, root length, dry weight, chlorophyll content, leaf area index (LAI) and crop yield[54,55]. The biochemical parameters like crude protein, dietary fiber, total carbohydrate and total sugar in *S. melongena* were determined following standard methods[51].

Extraction of heavy metals and their analysis

For the heavy metals analysis 10 ml of sugar mill effluent, 1.0 g of air dried soil or plants were taken in digestion tubes separately. For each sample 3ml of concentrate HNO_3 was added and digested in an electrically heated block for 1 hour at 145°C. To this mixture 4 ml of $HClO_4$ was added and heated to 240°C for 1 hour. The mixture was cooled and filtered through Whatman # 42 filter paper. The volume was made to 50ml by adding double distilled water and used for analysis. Metals were analyzed using an atomic absorption spectrophotometer (PerkinElmer, Analyst 800 AAS, GenTech Scientific Inc., Arcade, NY) following methods[51]. In this field study, the contamination factor (Cf) was used to determine the contamination of metals in the soil and *S. melongena* irrigated with sugar mill effluent. The Cf was calculated following formula[51,54].

Data analysis

Data were analyzed with SPSS (ver. 14.0, SPSS Inc., Chicago, Ill.). Data were subjected to one-way analysis of variance (ANOVA). Mean standard deviation and coefficient of correlation (r-value) of soil and crop parameters with sugar mill effluent concentrations were calculated with MS Excel (ver. 2013, Microsoft Redmond Campus, Redmond, WA) and graphs produced with Sigma plot (ver. 12.3, Systat Software, Inc., Chicago, IL).

Results and Discussion

Characteristics of sugar mill effluent

The physico-chemical and microbiological characteristics of the sugar mill effluent are presented in table 1. The ANOVA analysis of data showed that the values of effluent parameters were found to be significantly ($P<0.05/P<0.01$) different over the concentrations of sugar mill effluent. The most values of TDS, EC, Na^+, K^+, Ca^{2+}, Mg^{2+}, PO_4^{3-}, SO_4^{2}, TKN, Cd, Cr, Cu, Fe, Mn, Zn, SPC and MPN of the sugar mill effluent were recorded with absolute (100% concentration) sugar mill effluent. The sugar mill effluent was highly alkaline (pH 8.80). The alkaline nature of the sugar mill effluent might be due to the more contents of alkalis used in the sugar manufacturing process. The higher value of EC (6.20 dS cm^{-1}) in the sugar mill effluent is might be due to more ionic species like Na^+ (140.40 mg L^{-1}), K^+ (260.45 mg L^{-1}), Ca^{2+} (624.60 mg L^{-1}), Mg^{2+} (190.70 mg L^{-1}), PO_4^{3-} (110.45

Table 1: Physico-chemical and microbiological characteristics of sugar mill effluent.

Parameter	0 (Bore well water)	Sugar mill effluent concentration (%)					BIS for irrigation water
		20	40	60	80	100	
TDS (mg L^{-1})	240.50	298.70*	604.20**	897.80***	1140.34***	1520***	1900
EC (dS cm^{-1})	0.10	1.26*	2.52*	3.78**	5.10**	6.20***	--
pH	7.16	7.24ns	7.36ns	7.45ns	8.42ns	8.80ns	5.0-9.0
BOD (mg L^{-1})	5.10	78.56*	140.30**	334.87**	650.80**	960.88***	100
COD (mg L^{-1})	10.77	154.80*	296.50**	740.78**	1478.50**	1670.20***	250
Na^+ (mg L^{-1})	12.45	28.20*	57.10*	86.65**	120.44**	140.40***	--
K^+(mg L^{-1})	20.50	48.30*	98.40*	148.86**	196.20**	260.45***	--
Ca^{2+} (mg L^{-1})	40.10	120.50	240.20*	362.97**	485.90**	624.60***	200
Mg^{2+}(mg L^{-1})	16.40	35.80*	72.60*	105.80**	150.12**	190.70***	--
TKN (mg L^{-1})	25.60	32.45*	45.80*	78.96**	126.34**	172.60***	100
PO_4^{3-} (mg L^{-1})	0.06	16.50*	34.12*	52.66**	90.34**	110.45***	--
SO_4^{2-} (mg L^{-1})	80.75	110.24*	225.90*	336.78**	454.70**	575.90***	600
Fe (mg L^{-1})	2.34	3.70*	7.14*	12.20**	15.80**	20.44***	15.00
Cd (mg L^{-1})	0.08	1.45**	2.90**	4.38**	5.86**	8.34***	1.00
Cr (mg L^{-1})	0.01	0.25*	0.52**	0.76**	1.23**	1.56***	2.00
Cu (mg L^{-1})	1.38	1.64*	3.18*	4.84**	6.38**	8.24***	3.00
Mn (mg L^{-1})	1.12	1.25*	2.56*	3.79**	5.18**	6.88***	1.00
Zn (mg L^{-1})	1.68	1.98*	4.37*	6.78**	8.88**	12.23***	2.00
SPC(SPC ml^{-1})	3.97×10^3	6.90×10^6**	7.25×10^8**	5.93×10^{10}***	6.88×10^{12}***	9.23×10^{14}***	10000
MPN(MPN100 ml^{-1})	2.32×10^2	4.78×10^4**	8.56×10^6**	3.98×10^8***	7.12×10^{10}***	5.34×10^{12}***	5000

Least squares means; ns, *, ** non-significant or significant at $P<0.05$ or $P<0.01$ or $P<0.001$ level of ANOVA, respectively.

mg L^{-1}) and SO_4^{2-} (575.90 mg L^{-1}) present in the sugar mill effluent. The higher number of SPC (9.23×10^{14} SPC ml^{-1}) and MPN (5.34×10^{12} MPN100 ml^{-1}) in the sugar mill effluent are likely due to the more TDS (1520 mg L^{-1}), BOD (960.88 mg L^{-1}), COD (1670.20 mg L^{-1}) and TKN (172.60 mg L^{-1}) in the sugar mill effluent. The higher number of MPN is might be associated with the domestic sewage received from the staff quarters and offices located in the premises of the sugar mill. Kumar and Chopra[48] reported that the more numbers of SPC (7.34×10^{18} SPC ml^{-1}) and MPN (5.85×10^{16} MPN100 ml^{-1}) in the sugar mill effluent are due to the presence of more dissolved solids and organic matter in the effluent. Moreover, the higher contents of TDS and TKN in the sugar mill effluent support the more contents of Cd (7.28 mg L^{-1}), Cr (1.42 mg L^{-1}), Cu (8.05 mg L^{-1}), Fe (19.45 mg L^{-1}), Mn (6.42 mg L^{-1}) and Zn (10.34 mg L^{-1}) in the sugar mill effluent. Vijayaragavan et al.[38] also reported the higher contents of Cd (10.50 mg L^{-1}), Cr (3.80 mg L^{-1}), Cu (14.75 mg L^{-1}), Fe (22.85 mg L^{-1}), Mn (6.00 mg L^{-1}) and Zn (16.40 mg L^{-1}) in the sugar mill effluent.

Effects of sugar mill effluent on soil characteristics

Tables 2-4 show the ANOVA data on physico-chemical characteristics of the soil after treatment with different concentrations of the sugar mill effluent. At harvest of *S. melongena* (120 days after plantation), the ANOVA data indicated that season, concentrations of the sugar mill effluent and their interaction showed insignificant change (P>0.05) in the soil texture (loamy sand; 40% sand: 40% silt: 20% clay), BD and WHC (Table 2). The season, concentrations of the sugar mill effluent and their interaction with season showed significant (P<0.05/P<0.01) effect on OC, Na^+, K^+, Ca^{2+}, Mg^{2+}, TKN, PO_4^{3-}, SO_4^{2-}, Cd, Cr, Cu, Fe, Mn and Zn in both seasons (Tables 2-4). The season did not show significant (P>0.05) change in the EC and pH of the soil but the concentrations of the sugar mill effluent and their interaction with season showed significant (P<0.05) change in the EC and pH of the soil (Table 2).

Table 2: ANOVA for effect of sugar mill effluent (SME) on soil characteristics.

Source		WHC	BD	EC	pH	OC	TKN
Season (S)	ns	ns	ns	ns	*	*	
SME concentration (C)	ns	ns	*	*	**	**	
Interaction S × C	ns	ns	*	*	**	**	

SME- sugar mill effluent; ns, *, ** non-significant or significant at P<0.05 or P<0.01 level of ANOVA, respectively.

Table 3: ANOVA for effect of sugar mill effluent on cations in soil.

Source	Na^+	K^+	Ca^{2+}	Mg^{2+}	PO_4^{3-}	SO_4^{2-}
Season (S)	*	*	*	*	*	*
SME concentration (C)	*	*	**	*	**	*
Interaction S × C	*	**	**	**	**	*

SME- sugar mill effluent; *, ** significant at P<0.05 or P<0.01 level of ANOVA, respectively.

Table 4: ANOVA for effect of sugar mill effluent on metals in soil.

Source		Cd	Cr	Cu	Fe	Mn	Zn
Season (S)	*	*	*	*	ns	*	
SME concentration (C)	**	**	**	**	*	**	
Interaction S × C	**	**	**	**	**	**	

SME- sugar mill effluent; *, ** significant at P<0.05 or P<0.01 level of ANOVA, respectively.

Table 5: Effects of sugar mill effluent concentration and season interaction on physico-chemical characteristics of soil used in the cultivation of *S. melongena* in both seasons.

Season × %SME		EC (dS m^{-1})	pH	OC (mg kg^{-1})	Na^+ (mg kg^{-1})	K^+ (mg kg^{-1})	Ca^{2+} (mg kg^{-1})	Mg^{2+} (mg kg^{-1})
Rainy	0	1.18	7.52	0.45	22.48	150.36	45.98	38.60
	20	1.68ns	7.64ns	2.69*	28.94*	166.74ns	55.67*	48.64*
	40	3.76*	7.84ns	5.78**	54.77*	195.68**	75.94**	65.72**
	60	5.56*	7.98*	7.94**	68.95*	244.80**	86.94**	86.30**
	80	7.66*	8.12*	9.67**	82.48**	275.94*	136.85**	102.14*
	100	9.86**	8.64*	12.94**	90.26**	298.64*	186.95**	125.94*
Summer	0	1.19	7.54	0.48	23.48	155.36	46.80	40.36
	20	1.86ns	7.75ns	2.95*	30.25*	175.40ns	62.40*	58.64*
	40	4.52*	7.94ns	6.15**	56.84*	182.69*	86.37**	75.94**
	60	6.32*	8.15*	8.56**	78.48*	230.84*	115.61**	98.45**
	80	9.47*	8.38*	10.36***	85.49**	264.84**	152.69***	124.60***
	100	11.20**	8.84*	14.58***	95.48**	305.90**	205.64***	138.95***

Least squares means analysis; SME- sugar mill effluent; ns, *, ** non-significant or significant at P<0.05 or P<0.01 or P<0.001 level of ANOVA, respectively.

Table 5: Effects of sugar mill effluent concentration and season interaction on physico-chemical characteristics of soil used in the cultivation of *S. melongena* in both seasons.

Season × %SME		TKN (mg kg^{-1})	PO_4^{3-} (mg kg^{-1})	SO_4^{2-} (mg kg^{-1})	Cd (mg kg^{-1})	Cr (mg kg^{-1})	Cu (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})
Rainy	0	32.60	24.50	60.20	0.42	0.32	1.18	1.69	0.52	0.63
	20	74.85**	38.60*	86.94*	1.05*	0.68*	3.69*	5.58*	2.15*	1.95*
	40	162.40**	58.90*	142.63**	2.75*	1.06**	5.94*	7.84**	4.60*	3.94*
	60	280.20**	84.75**	170.64**	4.64**	1.44**	8.67**	9.68**	6.91*	7.09**
	80	340.70**	115.64**	206.94**	5.96**	2.64**	10.94***	12.94**	7.94**	10.95***
	100	428.96*	137.83**	234.80**	7.33**	2.96**	12.88***	16.94**	10.62***	13.94***
Summer	0	34.26	25.64	62.32	0.43	0.32	1.19	1.72	0.53	0.64
	20	82.94**	46.50*	92.67*	1.14*	1.12*	3.95*	6.88*	2.85*	2.26*
	40	195.75**	70.94*	156.39**	2.96*	1.65**	6.42*	8.67**	5.38*	5.64*
	60	296.80**	102.60**	185.94**	5.12**	1.95**	9.68**	10.64**	7.61**	9.67**
	80	368.88***	130.48***	226.55***	6.44***	2.75***	12.94***	14.69***	10.26***	13.54**
	100	445.80***	154.66***	243.80***	8.15***	3.15***	14.20**	18.85***	12.39**	15.32***

Least squares means analysis; SME- sugar mill effluent; ns, *, ** non-significant or significant at $P<0.05$ or $P<0.01$ or $P<0.001$ level of ANOVA, respectively.

During the present study, irrigation with 100% concentration of the sugar mill effluent showed the most increase in the EC, OC, Na^+, K^+, Ca^{2+}, Mg^{2+}, TKN, PO_4^{3-}, SO_4^{2-}, Cd, Cr, Cu, Fe, Mn and Zn in both seasons (Table 5). The values of WHC and BD of the sugar mill effluent irrigated soil were insignificantly changed with different concentrations of the sugar mill effluent in both the seasons. The WHC and BD of the soil were reduced from their control values 44.60% and 1.42 gm cm^{-3} to 42.80, 42.96% and 1.41 gm cm^{-3}, respectively due to irrigation with 100% concentration of the sugar mill effluent. The WHC is related to the number and size distribution of soil pores, soil moisture content, textural class, and structure, salt content and organic matter. The BD of soil changes with the application of organic manure to soil that substantially modifies, and lowers the soil bulk density. It is used for determining the amount of pore space and water storage capacity of the soil. The organic matter supplied through the sugar mill effluent can lower the BD and WHC as earlier reported by Maliwal et al.[30]. The findings of the present study are also in accordance to Kumar and Chopra[54] who reported that the higher contents of OC in sewage effluent lowered the BD and WHC of the soil.

The results showed that 40 to 100% concentrations of the sugar mill effluent significantly ($P<0.05/P<0.01/P<0.001$) affected EC, OC, Na^+, K^+, Ca^{2+}, Mg^{2+}, TKN, PO_4^{3-}, SO_4^{2-}, Cd, Cr, Cu Fe, Mn and Zn of the soil used for the cultivation of *S. melongena* in both seasons (Table 5). The pH (8.64 and 8.84) of the soil was found more alkaline with 100% treatment of the sugar mill effluent and it is likely due to the alkaline nature (pH 8.80) of the sugar mill effluent. The higher values of EC of the sugar mill effluent irrigated soil might be due to the presence of more cations and anions in the soil suspension. Moreover, the EC, OC, Na^+, K^+, Ca^{2+}, Mg^{2+}, TKN, PO_4^{3-}, SO_4^{2-}, Cd, Cr, Cu, Fe, Mn and Zn of the soil were noted to be positively correlated with different concentrations of the sugar mill effluent in both seasons (Table 6). Thus there was gradual build up of EC, OC, Na^+, K^+, Ca^{2+}, Mg^{2+}, TKN, PO_4^{3-}, SO_4^{2-}, Cd, Cr, Cu, Fe, Mn and Zn of the soil due to the concentrations of the sugar mill effluent.

Table 6: Coefficient of correlation (r) between sugar mill effluent and soil characteristics

Sugar mill effluent /soil characteristics	Season	r - value
Sugar mill effluent versus soil EC	Rainy	+0.94
	Summer	+0.95
Sugar mill effluent versus soil pH	Rainy	-0.90
	Summer	-0.92
Sugar mill effluent versus soil OC	Rainy	+0.97
	Summer	+0.98
Sugar mill effluent versus soil Na^+	Rainy	+0.92
	Summer	+0.94
Sugar mill effluent versus soil K^+	Rainy	+0.88
	Summer	+0.90
Sugar mill effluent versus soil Ca^{2+}	Rainy	+0.94
	Summer	+0.96
Sugar mill effluent versus soil Mg^{2+}	Rainy	+0.82
	Summer	+0.84
Sugar mill effluent versus soil TKN	Rainy	+0.96
	Summer	+0.98
Sugar mill effluent versus soil PO_4^{3-}	Rainy	+0.95
	Summer	+0.96
Sugar mill effluent versus soil SO_4^{2-}	Rainy	+0.92
	Summer	+0.94
Sugar mill effluent versus soil Fe	Rainy	+0.95
	Summer	+0.96
Sugar mill effluent versus soil Cd	Rainy	+0.90
	Summer	+0.92
Sugar mill effluent versus soil Cr	Rainy	+0.86
	Summer	+0.88
Sugar mill effluent versus soil Cu	Rainy	+0.96
	Summer	+0.98
Sugar mill effluent versus soil Mn	Rainy	+0.94
	Summer	+0.95
Sugar mill effluent versus soil Zn	Rainy	+0.96
	Summer	+0.98

Rakkiyappan et al.[55] reported that sugar mill effluent amendments increased EC, pH, total organic carbon (TOC), total Kjeldahl nitrogen (TKN), and available phosphorus, exchangeable Na, K, Ca and Mg in soil. The soil pH is an important parameter as many nutrients are available to plants only within a particular pH range. A pH range of 6.0 to 9.4 enhances nutrient availability for plants, and a pH below 6.0 and above 8.8 inhibits the availability of nutrients for plants[56-58]. In the present study pH of the soil was ranged 8.58-8.80 with 100% treatment of the sugar mill effluent which makes the various soil nutrients available to the plants.

The contents of TKN and OC in the soil irrigated with sugar mill effluent were recorded higher than the control soil. The more organic carbon in sugar mill effluent irrigated soil might be due to the high organic nature of the sugar mill effluent. Kumar and Chopra[46] found higher organic content in the soil irrigated with sugar mill effluent. The higher values of Na^+, K^+, Ca^{2+}, Mg^{2+}, PO_4^{3-} and SO_4^{2-} in the soil treated with sugar mill effluent were likely due to the more contents of Na^+, K^+, Ca^{2+}, Mg^{2+}, PO_4^{3-} and SO_4^{2-} in the sugar mill effluent. The varied accumulation of the Na ($3s^1$), K ($4s^1$), Ca ($4s^2$), Mg ($3s^2$), P ($3p^3$); S ($3p^4$), Fe ($3d^6$), Cd ($4d^{10}$), Cr ($3d^5$), Cu ($3d^{10}$), Mn ($3d^5$) and Zn ($3d^{10}$) in the soil after effluent irrigation is likely due to the electrons present in their s, p and d orbitals which determine their stability, reactivity, oxidation and reduction in the aqueous environment.

The contents of heavy metals, Cd, Cr, Cu, Fe, Mn and Zn in the soil were increased with the increase in the concentrations of the sugar mill effluent. The contamination factor (Cf) of the heavy metals indicated that Zn (22.13 and 23.94) was highest while Cr (9.25 and 9.84) was lower in both seasons after treatment with 100% sugar mill effluent. The Cf of heavy metals were in the order of Zn>Mn>Cd>Cu>Fe>Cr after irrigation with sugar mill effluent in both seasons (Figure 1). Although, the contents of heavy metals except Cd (6.0 mg kg^{-1}) were found below the maximum levels permitted for the Cr (10.0 mg kg^{-1}), Cu (270 mg kg^{-1}) and Zn (600 mg kg^{-1}) for the soils in India[59]. However, there was a gradual build up of these metals in the sugar mill effluent irrigated soil. Thus, sugar mill effluent irrigation increased the nutrients and metals in the soil used for the cultivation of *S. melongena*.

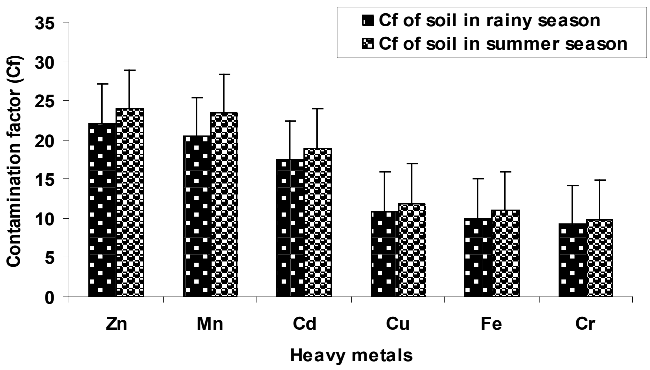

Figure 1: Contamination factor of heavy metals in soil after irrigation with sugar mill effluent. Error bars are standard error of the mean.

Effect of sugar mill effluent on seed germination of *S. melongena*

The percent seed germination of *S. melongena* after irrigation with sugar mill effluent is shown in figure 2. During the present study, most seed germination (86.00 and 88.00%) of *S. melongena* was observed with 60% concentration of the sugar mill effluent, while the least seed germination (80.00% and 82%) was noted with 100% concentration of the sugar mill effluent (Figure 2). The seed germination of *S. melongena* was noted to be negatively correlated (r = -0.56 and -0.58) with different concentrations of the sugar mill effluent in both the seasons. At germination stage, ANOVA indicated that seasons showed insignificant (P>0.05) effect on the seed germination of *S. melongena* and relative toxicity (RT) against the seed germination while sugar mill effluent concentrations and their interaction with seasons showed significant (P<0.05) effect on the seed germination of *S. melongena* and RT of the sugar mill effluent against the seed germination (Table 7). The findings are in agreement with Chopra et al.[53] who reported that the germination of okra (*Abelmoschus esculentus* L.) was decreased with the increase in the concentration of distillery effluent. In the present study, the higher concentrations (60% to 100%) of the sugar mill effluent did not support the seed germination of *S. melongena*. Kumar and Chopra[9] also reported that the germination of French bean (*Phaseolus vulgaris* L.) was decreased when the concentration of effluent increased. The higher concentrations (i.e. 80% and 100%) of the sugar mill effluent lowered the seed germination of *S. melongena*. It may be likely due to the presence of more contents of salts and heavy metals in the sugar mill effluent at higher concentrations of sugar mill effluent. The most RT (103.24% and 104.86%) of the sugar mill effluent against seed germination of *S. melongena* was recorded with 100% concentration of the sugar mill effluent (Figure 3). Moreover the RT was found to be significantly (P<0.05) and positively correlated (r = +0.64) with different concentrations of the sugar mill effluent.

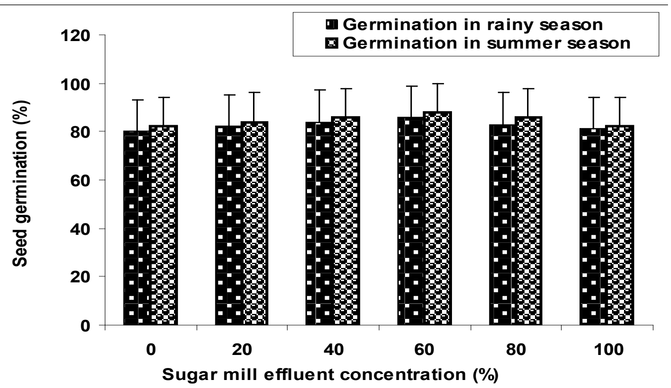

Figure 2: Seed germination of *S. melongena* after irrigation with sugar mill effluent. Error bars are standard error of the mean.

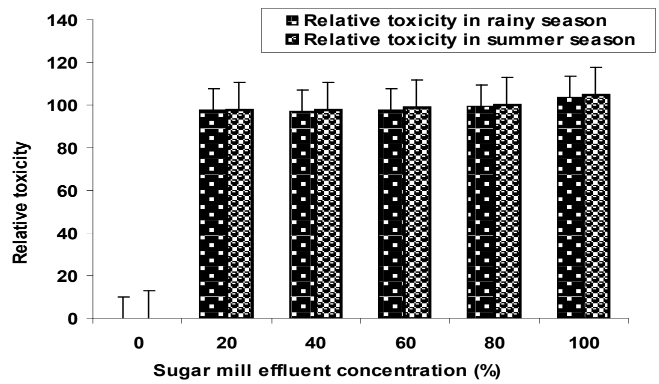

Figure 3: Relative toxicity of sugar mill effluent against seed

Table 7: ANOVA for effects of sugar mill effluent on different parameters of *S. melongena*.

Source	Seed germination	Relative toxicity	Plant height	Root length	Chlorophyll content	LAI	Dry weight	CY/plant
Season (S)	ns	ns	ns	ns	ns	ns	ns	ns
SME concentration (C)	*	*	*	ns	*	*	ns	*
Interaction S × C	*	*	*	ns	*	*	ns	*

SME- Sugar mill effluent; ns, *, non-significant or significant at P<0.05 level of ANOVA, respectively.

germination of *S. melongena*. Error bars are standard error of the mean.

Effect of sugar mill effluent on vegetative growth of S. *melongena*

Table 7 shows the ANOVA data for the effects of season, concentrations of the sugar mill effluent and their interaction on the attributes of the vegetative growth stage of *S. melongena*. The ANOVA indicated that concentrations of the sugar mill effluent and their interaction with season significantly (P<0.05) affected the plant height, chlorophyll content, and LAI of *S. melongena* in both the seasons. The season showed insignificant effect (P>0.05) on the vegetative growth attributes of *S. melongena*. Moreover season, concentrations of the sugar mill effluent and their interaction did not show significant effect on the root length of *S. melongena*.

In the present study, at vegetative growth at 75 days the maximum plant height (134.67 and 148.90 cm), root length (19.14 and 21.45 cm), dry weight (1264.50 and 1295.80 g), chlorophyll content (3.84 and 4.15 mg./g.f.wt) and LAI/plant (3.45 and 3.64) of *S. melongena* were observed with 60% concentration of the sugar mill effluent in both the seasons. The minimum plant height (107.55 and 112.76 cm), root length (13.87 and 14.23 cm), dry weight (880.24 and 896.90 g), chlorophyll content (3.12 and 3.18 mg./g.f.wt) and LAI/plant (3.24 and 3.30) of *S. melongena* were observed with control, while the moderate plant height (120.40 and 128.70 cm), root length (15.65 and 16.12 cm), dry weight (955.34 and 974.55 g), chlorophyll content (3.35 and 3.40 mg./g.f.wt) and LAI/plant (3.30 and 3.37) of *S. melongena* were noted with 100% concentration of the sugar mill effluent in both seasons.

During the present study, plant height, root length, dry weight, chlorophyll content and LAI/plant of *S. melongena* were noted to be positively correlated with different concentrations of the sugar mill effluent in both the seasons (Table 8). Prabhu et al.[34] reported that the growth of *S. melongena* was decreased when the concentration of wastewater effluent increased. Likewise, Kumar and Chopra[9] reported that the maximum vegetative growth attributes like plant height, root length, dry weight, chlorophyll content and leaf area index of French bean (*Phaseolus vulgaris* L.) was noted with 60% concentration of the sugar mill effluent. Furthermore, the growth of P. vulgaris was decreased with the increase in sugar mill effluent concentration from 60% to 100%. The findings were also supported by Fukuoka et al.[35] who reported that the growth of eggplant was decreased due to the application of more concentration of waste effluent.

Table 8: Coefficient of correlation (r) between sugar mill effluent and *S. melongena*.

Sugar mill effluent / *S. melongena*	Season	r - value
Sugar mill effluent versus plant height	Rainy	+0.45
	Summer	+0.48
Sugar mill effluent versus root length	Rainy	+0.27
	Summer	+0.28
Sugar mill effluent versus dry weight	Rainy	+0.36
	Summer	+0.39
Sugar mill effluent versus chlorophyll content	Rainy	+0.54
	Summer	+0.56
Sugar mill effluent versus LAI	Rainy	+0.52
	Summer	+0.54
Sugar mill effluent versus crop yield/plant	Rainy	+0.42
	Summer	-0.44
Sugar mill effluent versus Cd	Rainy	+0.90
	Summer	+0.92
Sugar mill effluent versus Cr	Rainy	+0.74
	Summer	+0.76
Sugar mill effluent versus Cu	Rainy	+0.96
	Summer	+0.98
Sugar mill effluent versus Fe	Rainy	+0.94
	Summer	+0.96
Sugar mill effluent versus Mn	Rainy	+0.92
	Summer	+0.94
Sugar mill effluent versus Zn	Rainy	+0.96
	Summer	+0.98
Sugar mill effluent versus crude proteins	Rainy	+0.52
	Summer	+0.54
Sugar mill effluent versus dietary fiber	Rainy	+0.64
	Summer	+0.68
Sugar mill effluent versus total carbohydrates	Rainy	+0.60
	Summer	+0.64
Sugar mill effluent versus total sugar	Rainy	+0.56
	Summer	+0.58

The results showed that the vegetative growth of *S. melongena* was decreased at higher (i.e. 80% to 100%) concentrations of the sugar mill effluent. It might be due to the presence of more contents of the heavy metals in the higher concentrations of sugar mill effluent, which lowered the plant height, root length, dry weight, chlorophyll content and LAI/plant of *S. melongena*. Vegetative growth is concerned with the development of new shoots, leaves and leaf area[41]. The maximum plant height, root length, dry weight and LAI/plant of *S. melongena* were noted with 60% concentration of the sugar mill effluent, and it may be likely due to the maximum uptake of nitrogen (N), phosphorus (P) and potassium (K) by *S. melongena* plants. The improvement of vegetative growth may be attributed to the role of K in the nutrient and sugar translocation in the plants and turgor pressure in the plant cells. It is also involved in the cell enlargement and in triggering young tissue or mersitematic growth[31,33]. The chlorophyll content was found higher due to the use of 60% concentration of the sugar mill effluent in both seasons, and is likely due to Fe, Mg and Mn contents in the sugar mill effluent, which are associated with chlorophyll synthesis[34,54]. Thus, 60% concentration of the sugar mill effluent contains optimum contents of nutrients required for the maximum vegetative growth of *S. melongena*.

Effect of sugar mill effluent on maturity of *S. melongena*

The ANOVA data indicated that the crop yield/plant of *S. melongena* were not affected by seasons, sugar mill effluent concentrations and their interaction (Table 7). Furthermore, the crop yield/plant of *S. melongena* was found to be positively correlated with all concentrations of the sugar mill effluent in both the seasons (Table 8). At maturity stage (120 days after sowing), the maximum yield/plant (1465.80 and 1525.65 g) at I harvest, (1848.90 and 1886.60 g) at II harvest and (1290.55 and 1366.77 g) at III harvest of *S. melongena* were recorded with 60% concentration of the sugar mill effluent in both the seasons. The least crop yield/plant (1018.87 and 1058.43 g) at I harvest, (1135.66 and 1189.80 g) at II harvest and (1098.50 and 1108.90 g) at III harvest of *S. melongena* were recorded with the control while moderate crop yield/plant (1230.10 and 1298.20 g) at I harvest, (1566.54 and 1650.12 g) at II harvest and (1168.30 g and 1257.42 g) of *S. melongena* at III harvest were recorded with 100% treatment of the sugar mill effluent in both the seasons. At maturity stage the 60% concentration of the sugar mill effluent favored crop yield of *S. melongena*. This is likely due to the presence of optimal contents of K, Fe, Mg and Mn in the 60% concentration of the sugar mill effluent. Moreover, the higher concentrations (i.e. 80% to 100%) of the sugar mill effluent lowered the crop yield of *S. melongena*. The reduction in the crop yield is likely due to the presence of higher contents of heavy metals, which reduced the uptake of nutrients by *S. melongena* plants.

The role of K, Fe, Mg and Mn at maturity is important and associated with synthesis of chlorophyll, and enhances the crop yield[48,51]. The K, Fe, Mg and Mn contents could enhance the yield of eggplants (*S. melongena*) as reported by Fukuoka et al.[35]. Kumar et al.[39] reported that the maximum crop yield of black gram (*Vigna mungo* L.) was noted with 40% concentration of effluent. Moreover, the crop yield of *V. mungo* was decreased with the increase in effluent concentration from 60% to 100%.

Effect on heavy metals and biochemical components in *S. melongena*

Tables 9 and 10 show the ANOVA data for the effects of season, concentrations of the sugar mill effluent and their interaction on the contents of heavy metals and biochemical components in *S. melongena*. The ANOVA indicated that season, concentrations of the sugar mill effluent and their interaction significantly ($P<0.05/P<0.01$) affected the contents of heavy metals and biochemical components in *S. melongena*. The 20% to 100% concentrations of the sugar mill effluent showed significant ($P<0.05/P<0.01$) effect on Cd, Cr, Cu, Fe, Mn and Zn, crude protein, dietary fiber, total carbohydrate and total sugar in *S. melongena*. The contents of Cd, Cr, Cu, Fe, Mn, Zn, crude protein, dietary fiber, total carbohydrate and total sugar were found to be positively correlated with all concentrations of the sugar mill effluent in both the seasons (Table 8). The most contents of Cd, Cr, Cu, Fe, Mn and Zn in *S. melongena* were recorded with 100% treatment of the sugar mill effluent (Figures 4, 5). It might be due to the presence of higher contents of heavy metals in 100% treatment of the sugar mill effluent, which added more metals in the soil environment. The accumulation of Cr, Cu, Fe, Mn and Zn except Cd in *S. melongena* was noted below the permissible limit of FAO/WHO standards for Cd (0.20 mg Kg^{-1}), Cr (2.30 mg Kg^{-1}), Cu (40.00 mg Kg^{-1}) Fe (80.00 mg Kg^{-1}) and Zn (60.00 mg Kg^{-1})[60]. The contamination factor (Cf) of heavy metals was affected in both seasons. The Cf of the metals was noted in the order of Fe>Mn>Cu>Zn>Cd>Cr in *S. melongena* after application of sugar mill effluent (Figure 6). The highest contamination factor was noted for Fe (12.68 and 13.33), while the least was found for Cr (6.72 and 7.42) in *S. melongena* with 100% concentration of the sugar mill effluent in both the seasons. The diverse accumulation of these metals might be due to the number of electrons in the d-levels of the atom. Although, metals with completely filled d orbitals such as ($3d^{10}$ of Cu and Zn and $4d^{10}$ of Cd) may be least incorporated compared to ($3d^5$ of Cr and Mn) into the *S. melongena* plants due to their lower reactivity and more stability imparted by the completely filled d orbitals. The lower reactivity and stability of these metals reduce the rate of various reactions such as absorption, ionic exchange, redox reactions, precipitation and dissolution through which plants take metals from the soils. However, the bioavailability of these metals might be increased due to the ionization in the aqueous phase in the soil which increases their reactivity and instability as earlier reported by Kumar et al.[39]. The results showed that the contents of these metals were more at 80% to 100% concentrations of the sugar mill effluent, and likely inhibited growth of *S. melongena*. The 60% treatment of the effluent favored vegetative growth and maturity of S*S. melongena*. This is likely due to optimal uptake of these metals by *S. melongena* plants, which supports various biochemical and physiological processes of the *S. melongena* plants. The results are in the conformity of Kumar and Chopra[25] who reported the accumulation of metals in the order of Fe>Zn>Cd>Cu>Cr>Pb in *Vicia faba* after irrigated with sugar mill effluent. Nunome et al.[33] also reported the contamination of Cd, Cu, Cr, Zn and Pb in soil and *S. melongena* after application of sugar mill effluent. During the present study, maximum contents of crude protein, dietary fiber, total carbohydrate and total sugar were noted with 60% concentration of the sugar mill effluent (Figures 7-10). The contents of crude protein, dietary fiber, total carbohydrate and total sugar were decreased from 80% to 100% concentrations of the sugar mill effluent. The findings are in agreement with Kumar and Chopra[9,46] who reported that the contents of total carbohydrate, crude protein and crude fiber in French bean (*Phaseolus vulgaris*) and spinach (*Spinacia oleracea* L.) were decreased with the increase in the concentration of sugar mill effluent, respectively when the effluents were used for fertigation.

Table 9: ANOVA for effects of sugar mill effluent on metals in *S. melongena*

Source	Cd	Cr	Cu	Fe	Mn	Zn
Season (S)	*	*	*	*	*	*
SME concentration (C)	*	*	**	**	*	**
Interaction S × C	*	*	**	**	*	**

SME- sugar mill effluent; .ns, *, ** non-significant or significant at P<0.05 or P<0.01 level of ANOVA, respectively.

Table 10: ANOVA for effects of sugar mill effluent on biochemical components of *S. melongena*

Source	Crude proteins	Dietary fiber	Total carbohydrates	Total sugar
Season (S)	*	*	*	*
SME concentration (C)	*	**	**	**
Interaction S × C	*	**	**	**

SME- sugar mill effluent; ns, *, ** non-significant or significant at P<0.05 or P<0.01 level of ANOVA, respectively.

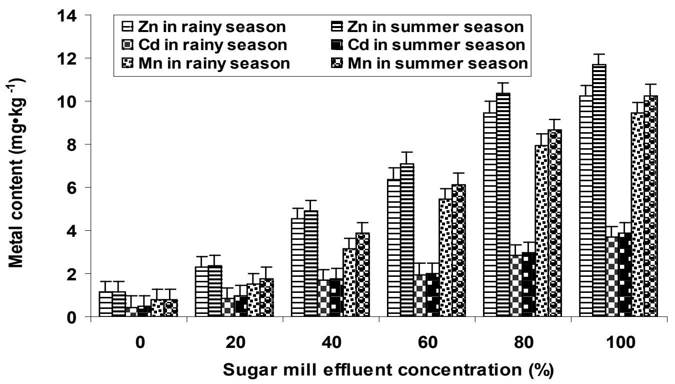

Figure 4: Contents of Zn, Cd and Mn in *S. melongena* after irrigation with sugar mill effluent. Error bars are standard error of the mean.

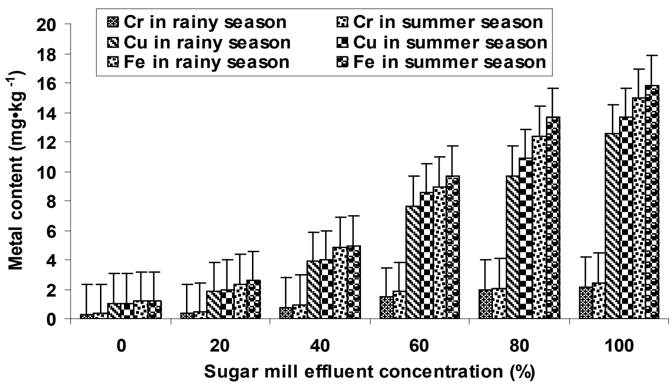

Figure 5: Contents of Cr, Cu and Fe in *S. melongena* after irrigation with sugar mill effluent. Error bars are standard error of the mean.

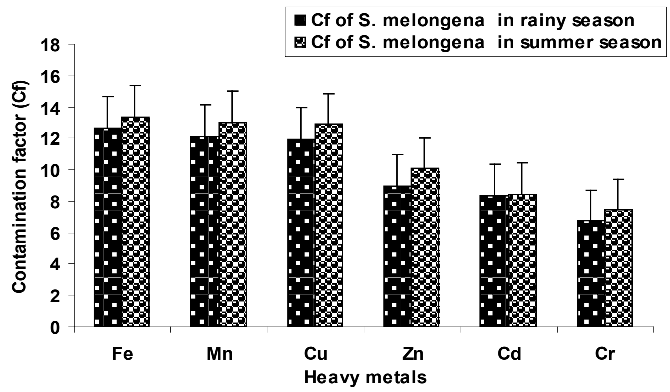

Figure 6: Contamination factor of heavy metals in *S. melongena* after irrigation with sugar mill effluent. Error bars are standard error of the mean.

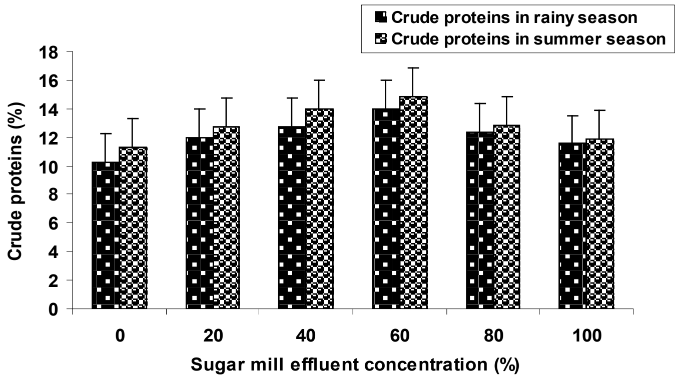

Figure 7: Crude proteins in *S. melongena* after irrigation with sugar mill effluent. Error bars are standard error of the mean.

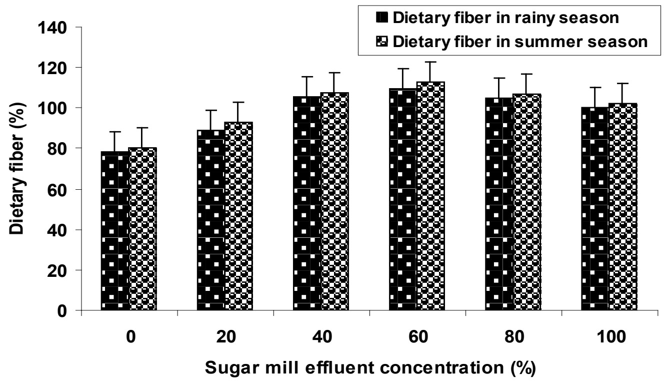

Figure 8: Dietary fiber in *S. melongena* after irrigation with sugar mill effluent. Error bars are standard error of the mean.

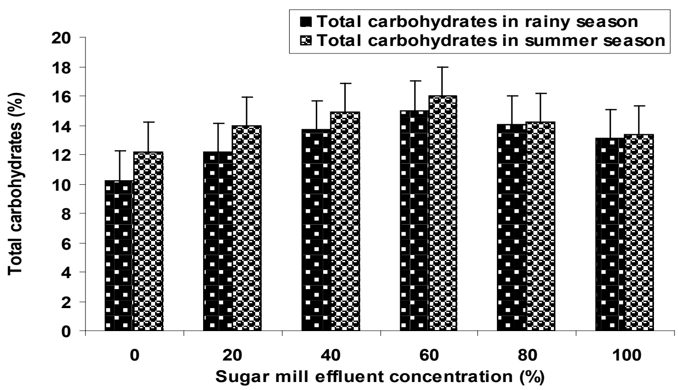

Figure 9: Total carbohydrates in *S. melongena* after irrigation with sugar mill effluent. Error bars are standard error of the mean

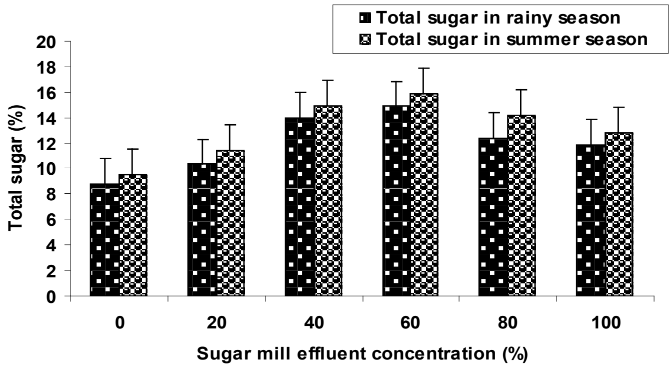

Figure 10: Total sugar in *S. melongena* after irrigation with sugar mill effluent. Error bars are standard error of the mean.

Conclusion

This study concluded that the concentrations of the sugar mill effluent viz. 0% (control), 20%, 40%, 60%, 80% and 100% significantly (P<0.05/P<0.01) affected the EC, pH, OC, Na^+, K^+, Ca^{2+}, Mg^{2+}, TKN, PO_4^{3-}, SO_4^{2-}, Cd, Cr, Cu, Fe, Mn and Zn of the soil used for the cultivation of *S. melongena* in both seasons. The agronomic performance of *S. melongena* was gradually increased from 20 to 60% and decreased from 80% to 100% concentrations of the sugar mill effluent in both the seasons compared to controls. The maximum agronomical attributes at vegetative growth stage and maturity stage of *S. melongena* along with biochemical components like crude proteins, dietary fiber, total carbohydrates and total sugar in *S. melongena* were found with 60% concentration of the sugar mill effluent in both seasons. The contamination factor (Cf) of various metals were in the order of Zn>Mn>Cd>Cu>Fe>Cr for the soil and Fe>Mn>Cu>Zn>Cd>Cr for *S. melongena* in both seasons after irrigation with sugar mill effluent. It appears that sugar mill effluent can be used as a biofertigant after appropriate dilution (up to 60%) to improve yield of *S. melongena* crop. Further studies on the agronomic growth and changes in biochemical composition of *S. melongena* after sugar mill effluent irrigation are required.

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