Annals of Plant and Soil Research 21(3): 250-255 (2019)

Effect of sugar mill effluent on growth and biochemical parameters of marigold (*Tagetes erecta*)

TARUN KUMAR, AARTI AND AMITA MITTAL*

Department of Biotechnology, University Institute of Engineering & Technology, Kurukshetra University, Kurukshetra (Haryana)- 136119, India

Received: June, 2019; Revised accepted: July, 2019

ABSTRACT

A study was carried out in the Department of Biotechnology, Univeristy Institute of Engineering and Technology, Kurukshetra University Kurukshetra, Haryana during 2018 to study the effects of diluted sugar mill effluent on growth and biochemical constituents of Tagetes erecta L. Pot culture experiment was conducted on seasonal marigold plants by irrigating with different concentrations of sugar mill effluent (25%, 50%, and 75% v/v). All pots were irrigated with 100 ml of respective concentration of effluent at an interval of 24 hours up to 32 days. The data pertaining to plant growth, chlorophyll, soluble protein, total soluble sugar, proline content and malondialdehyde content was recorded at an interval of 8 days. Plants irrigated with diluted sugar mill effluent (25% v/v) have shown a significant increase in height (24 cm), number of leaves (145) and number of branches (14) as compared to plants irrigated with distilled water. Various concentrations of sugar industry effluent had negative effect on the chlorophyll content and maximum values of chlorophyll-a (12.02 mg/l), chlorophyll-b (12.56 mg/l) and total chlorophyll (24.58mg/l) were recorded in plants irrigated with distilled water (control).Effluent at 50% concentration produced plants with highest protein content (35.5 µg/l). Proline (18 µg/ml), sugar (4.6 mg/ml) and malondialdehyde (0.71 nmol.cm^{-1}) were highest in plants irrigated with 25% effluent concentration. Results of this study revealed that sugar industry effluent in diluted form had significantly beneficial effects on plant growth and can be used for agricultural activity.

Keywords: Sugar industry, effluent agricultural, biochemical, *Tagetes erecta*

INTRODUCTION

The sugar industry is contributing a major part in the development of Indian economy. India is the second largest sugarcane producers after Brazil in the world and on the first position when it comes to area in which it is cultivated. According to data from the Indian Sugar Mills Association, 35.5 million tons of this crop will be produced between October 2018 and September 2019. At present, India has almost 650 sugar (Mane *et al.,* 2015;Devi and Rani, 2017) mills producing 15 million tons of sugar and 13 million tons of molasses per year (Kumar and Chopra, 2014). This large scale production of sugar results into huge volume of effluent discharged mainly from floor waste water and condensate. Most of the sugar mill effluent released in to the rivers without adopting proper treatment methods. This inadequately treated effluent is heavily polluting soil and water resources which results dreadful health problems to the human, agriculture and aquatic ecosystem (Vaithiyanathan and Sundaramoorthy, 2017). The effluent contains large amount of various heavy metals. These metals affect physical and biochemical parameters of plant and degrade the nutritional value of soil (Ayyasamy *et al.,* 2008). In addition to the heavy metals; magnesium, phosphorus, calcium, nitrogen and potassium are also present in the effluent. These macro and micronutrients are of agricultural importance when present under permissible amounts (Pedreroa *et al.,* 2019). Disposal of the effluent has become a headache for population living around sugar mills. Farmers are using sugar mill effluent as a replacement for chemical fertilizers as it contains nutrients that are required for plant growth. Use of such effluent for agronomical activities can influence soil properties and agricultural crop growth. But on the other hand for farmers this practice is an alternate to the use of high cost chemical fertilizers and irrigation water, as it is available to them free of cost. Prolonged use of sugar mill effluent containing large amount of heavy metals may have harmful effects on the soil characteristics and crop. Preventive measures should be there to assess the amount of effluent suitable for agricultural activities without affecting soil properties, crop quality and production. This study was done to assess the effects of diluted effluent on plant growth and other chemical constituents.

MATERIAL AND METHODS

The samples of sugar mill effluent were collected in plastic containers prewashed with acid from the drainage point of Saraswati sugar mill, Yamuna nagar district, Haryana, India. A cold chain was maintained while transporting the effluent sample and stored under cold environment till further experimental activities. Various physicochemical parameters like pH, Temperature and Electrical conductivity were studied. Soil for the experiment was collected from agricultural land located around Kurukshetra University Kurukshetra. Soil was dried under sunlight and finely crushed to powder from.Pot experiment was conducted for 32 days during the plant season. Saplings were grown in pots containing 20kg soil irrigated with three effluent concentrations viz. 25%, 50% and 75% and distilled water (control). For each effluent concentration and control, three pots were prepared to authenticate the results. Physical parameters of plant such as height, leaves and branches were observed after an interval of 8 days. Any visible wilting, whitening or yellowing of leaves or any other plant part was also observed and noted. Chlorophyll was determined as per procedure of Hiscox and Israelstam, (1979). Bradford (1976) method was adopted for the protein estimation. Estimation of proline was done with the procedure of Bates *et al.* (1973). Soluble sugars were determined by adopting the method of Dey (1990).Freshly harvested plant leaves (300 mg) were homogenized thoroughly in 5% trichloro acetic acid and centrifuged at 12,000 rpm for 20 min at 25^0 C. 2 ml of the supernatant was mixed with to an equal volume of 0.5% TBA in 20% tri-chloro acetic acid and incubated at 95^0C for 25 min for development of orange color. The absorbance of the supernatant was measured at 532 nm. The amount of malondialdehyde (MDA) was calculated by using the formula (Heath and Packer, 1968):

MDA equivalents (nmol.cm^{-1}) = 1000[(Abs 532-Abs 600 nm)/155]

RESULTS AND DISCUSSION

Physico-chemical parameters:

The results on physico-chemical parameters of sugar industry effluent are summarized in Table 1.

Table 1: Physico-chemical parameters of sugar effluent

Physico-chemical parameters	Sugar mill effluent
pH	7.42
Electrical conductivity (dSm^{-1})	4.23
Temperature	24^0 C
Colour	Yellowish
Texture	Clay loam
Odour	Unpleasant

These results showed that the sugar mill effluent was slightly basic, yellowish in colour having high electrical conductivity. Large amounts of dissolved solids and suspended particles were also present in the effluent.

Plant studies: At 75% effluent concentration, plant height, number of leaves and branches increased initially but at the completion of experiment after 32 days there was adecrease in growth of *Tagetes erecta*along with whitening and wilting of leaves.As evident from results(table 2) sugar industry effluent at highest concentration (75%) has initially helped to increase the plant height but after 24 days plant height did not increase significantly. Data recorded after 32 days showed that plants irrigated with lowest concentration (25%) of effluent were the tallest with a height of 24.5 ± 0.62 cm. In a study Vaithiyanathan and Sundaramoorthy (2017) analyzed the effect of sugar mill effluent on African marigold and found that germination and growth parameters increased at lower (10%) concentration of sugar mill effluent and this morphological parameters gradually decreased with increasing effluent concentration. Increased plant growth at lower concentration may be due to permissible amount of nutrients. The lower concentration of effluent played the role of chemical fertilizers by providing adequate amount of essential growth regulators such as nitrogen, phosphorous as suggested by Augusthy and Annsherin (2001). Results of present study corroboratie with the findings of Srivastava *et al.* (2012) who studied ferti-irrigational effect of distillery effluent on *Solanum melongena* and found that 50 % concentration significantly increased plant height, leaf area, number of leaves and branches.

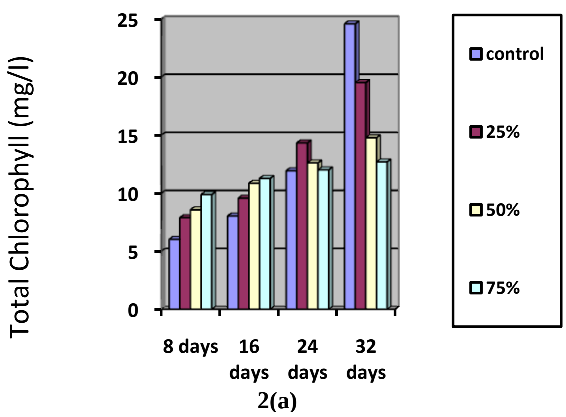
Table 2: Effect of different concentrations of sugar mill effluent on growth parameters of *Tagetes erecta*

Sugar mill effluent (Conc.)	Number of days			
	8	16	24	32
	Plant height			
Control	8.75 ± 0.18	13.5 ± 0.34	17.5 ± 0.35	19.75 ± 0.53
25%	10 ± 0.47	14.5 ± 1.03	20.5 ± 0.35	24.5 ± 0.62
50%	10.66 ± 0.72	15 ± 1.18	22 ± 1.41	23.5 ± 0.24
75%	11.83 ± 1.38	16.23 ± 0.51	22.5 ± 0.24	22.83 ± 1.66
	Number of leaves			
Control	55 ± 0.71	68.5 ± 1.06	103 ± 1.63	125 ± 2.36
25%	57 ± 4.19	83.66 ± 6.87	112.66 ± 5.58	145 ± 7.07
50%	69 ± 8.81	105 ± 10.80	145 ± 10.80	141.66 ± 4.91
75%	81 ± 4.50	134.66 ± 14.98	125 ± 2.36	106.66 ± 4.06
	Number of branches			
Control	6.33 ± 0.27	7.33 ± 0.27	8.66 ± 0.27	12 ± 0.47
25%	7 ± 0.82	8.33 ± 0.27	12.33 ± 0.27	14.33 ± 0.27
50%	7.66 ± 0.72	9.66 ± 0.98	12.66 ± 0.54	14 ± 0.47
75%	8 ± 0.94	10 ± 0.94	11.66 ± 0.27	12.66 ± 0.27

Similar pattern has been observed with the number of leaves produced by *Tagetes erecta*. Plants irrigated with 75%effluent concentration were having the highest number of leaves(134.66 ± 14.98) leaves after 16 days which decreased afterward (table 2). At the end of experiment after 32 days it was observed that at 25% effluent concentration plants developed 141.66 ± 4.91 leaves surpassing the plants growing on other concentrations and control.Results recorded at 32 days of experiment revealed that effluent at lowest concentration of 25% was the most effective in increasing the number of branches with highest count of 14.33 ± 0.27.The results of growth parameters observed in present study showed similarities with the previous work stating that the seed germination, total sugars, starch, reducing sugars and chlorophyll increased when tested on *Arachis hypogea* seedlings watered with 50% diluted textile waste in comparison to distilled water. The growth, biomass and nutritional status of *Eucalyptus* was better when watered with a mix of municipal and textile waste (Bhati and Singh, 2003). In another study, it was found that sawdust treated textile effluent at higher concentration effected plant growth negatively but the lower concentration (25%) was effective in increasing the growth of *Tagetes erecta* (Kaushik *et al.,* 2005).

Biochemical Parameters

Chlorophyll: Data(Figure 2) showed thatoverall effect of all the sugar effluent concentrations was negative on the chlorophyll-a, b and total chlorophyll content of Tagetes *erecta*.Amount of Chlorophyll-a (12.02 mg/l), chlorophyll-b (12.56 mg/l) and total chlorophyll (24.58) was highest in the plants raised on distilled water (control).It may be due tochlorophyll breakdown during stress or due to inhibition of chlorophyll biosynthesis (Ahmad *et al.,* 2006). Various abiotic stresses decrease the chlorophyll content in plants. Similarly, decrease in chlorophyll content was observed in *Cyamopsis tetragonoloba* when treated with plate making industry effluent (Selvaraj *et al.,* 2012).



2(a)

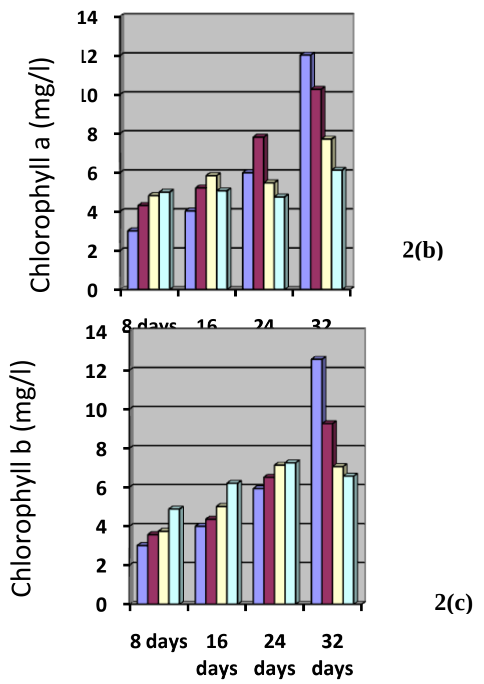


Fig.2: Effect of effluent on chlorophyll, of *Tagetes erecta*

Protein: Plants, watered with effluent (at 50% concentration) contained the highest protein content (35.5 µg/l) at all the intervals(figure 3). But after 16th day there was a drastic decrease in the amount of protein content, at all concentrations**.** Data recorded after 32 days at the completion of experiment showed that plant grown at 50% concentration were having 24 µg/l protein content. From this result it can be interpreted that prolonged use of sugar industry effluent lowered the protein content to some extant but still it is higher than the plants raised on other concentrations and distilled water (control). *Bhate and Singh* (2003) reported similar results.

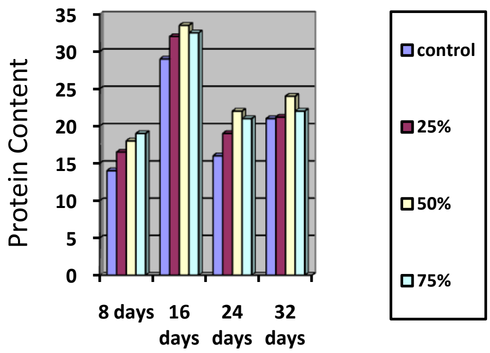


Figure 3: Effect of effluent on protein content (µg/ml) of *Tagetes erecta*

Proline: Proline content (14 µg/l) of plants irrigated with effluent concentration at 75% was highest when checked after 16 days which suddenly decreased thereafter. Data recorded after 32 days showed that plants watered with 25% concentration of sugar effluent were generating 18 µg/mlof proline which turned out to be significantly higher than plants raised on other concentrations and distilled water.Proline is an amino acid, which normally accumulates in almost all organisms when exposed to abiotic stress (Ericson and Alfinito, 1984).

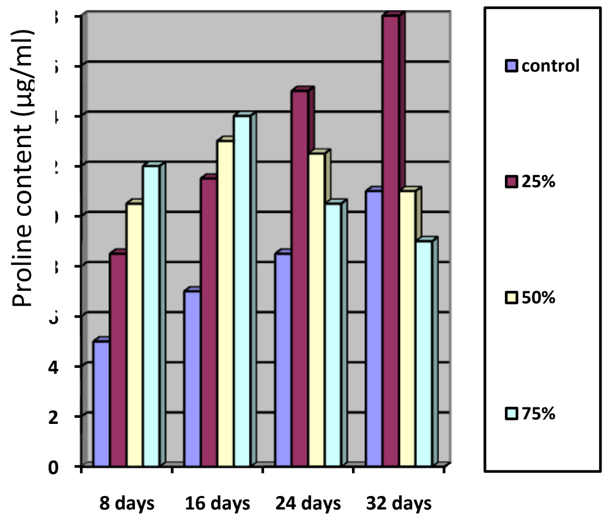


Figure 4: Effect of effluent on proline content (µg/ml) of *Tagetes erecta*

Sugar: Sugar content was higher in plants irrigated with different concentrations of effluent as compared to the plants raised on distilled water at all the intervals. After 32 days of treatment it was found thatlower concentration (25%) of effluent produced plants having the highest value of sugar content (4.6 mg/ml)(figure 5). In a study, Sarma and Sarma(2007) *grew Amellia sinensis*, *Aegle marmelos, Anthocephalus cadamba, Colocasia leaves* (Black) and *Lantana camara* on site contaminated with the area where the fertilizer industry effluent were released. The plants showed extreme reduction in biochemical parameters viz chlorophyll, protein and total soluble sugars.

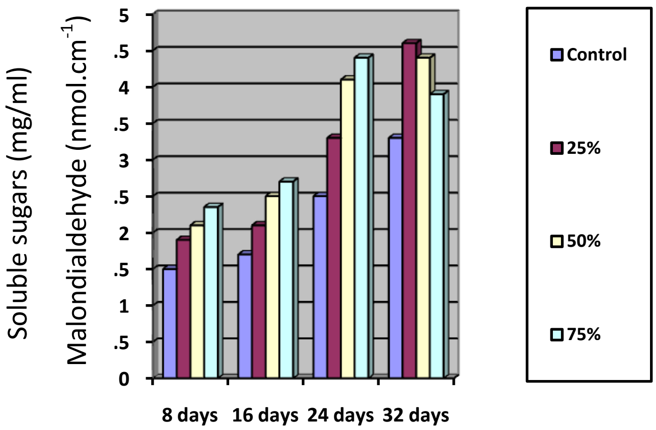


Figure 5: Effect of effluent on soluble sugars (mg/ml) of *Tagetes erecta*

MDA: Similar pattern have been observed in case of malondialdehyde content. Data recorded after 24 days showed that MDA content was highest in plants watered with 75% effluent concentration. Plants growing at higher concentrations (75% and 50%) have shown a decrease in malondialdehyde content after 32 days. Highest value was observed in plants irrigated with sugar mill effluent at lowest concentration (25%). Olorunfemi and Lolodi (2011) reported that cassava processing effluent increased MDA levels in *Allium* Increased MDA content in maize plants was reported by Hussain *et al.* (2013) at 25% sugar mill effluent cocentration.

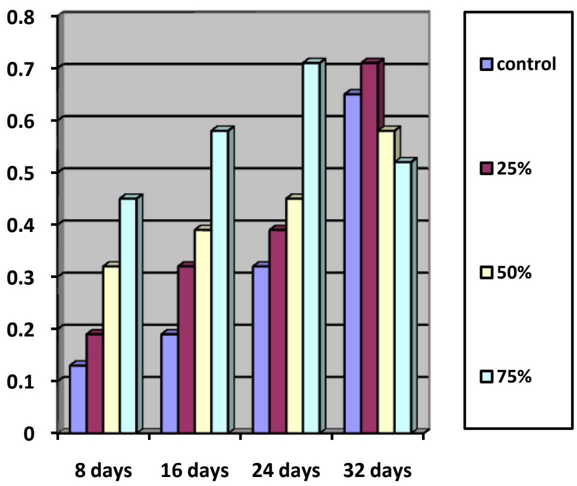


Fig: 6. Effect of effluent on malondialdehyde content (nmol.cm^{-1}) of *Tagetes erecta*

The exposition of *Tagetes erecta* to lower concentrations of sugar industry effluents helps to increase plant growth, proline content, proteins, soluble sugar content and malondialdehyde. But prolonged use of such effluent at higher concentration have severely affected the physical and biochemical parameters.There was a gradual decrease in biochemical parameters ofplants, when irrigated with various effluent concentrations (except 25%) as compared to the control. Effluent in diluted form at 25% concentration favoured the plant growth and increased the plant height, number of leaves, branches and biochemical parameters. Excess amount of inorganic nutrients and toxicity level of sugar industry effluent can be decreased by diluting it with water used for irrigation.

Finding of this study have revealed that the effluent after diluting up to 25 % can be used for irrigation as soil fertilizers forthe better growth and survival of the plant. Waste water of sugar industry can be reused for irrigation of plants which helps in better growth. Application of such effluent for agricultural activity may be sustainable and economical due to nutrient cycling and disposal of waste water. This study can be beneficial for farmers and environment as it provides a better solution to the wastewater disposal. More work can be done to observe long term effects of sugar mill effluent on plant flowering, productivity and survival.

ACKNOWLEDGMENTS

The authors are grateful to Director, UIET, Kurukshetra University, Kurukshetra, India, providing facilities for this research and TEQIP for financial support.

REFERENCES

Ahmad, P., Sharma, S. and Srivastava, P.S. (2006) Differential physio-biochemical responses of high yielding varieties of Mulberry (*Morusalba*) under alkalinity (Na_2CO_3) stress in vitro. *Physiology and molecular biology of plants* **12**: 59-66.

Augusthy, P.O. and Annsherin, M. (2001) Effect of factory effluent on seed germination and seedling growth of Vigna radiatus (L.). *Journal of Environtal Research* **22**(2):137–139

Ayyasamy, P.M., Yasodha, R., Kumar, R.S., Lakshmanaperumalsamy, P., Rahman P.K.S.M. and Lee, S. (2008) Impact of sugar factory effluent on the growth and biochemical characteristics of terrestrial and aquatic plants. *Bulletin of environmental contamination and toxicology* **81**:449–454.

Bates, L., Waldren, R.P. and Teare, I.D. (1973) Rapid determination of free proline for water-stress studies. *Plant and Soil* **39**: 205-207.

Bhati, M. and Singh, G. (2003) Growth and mineral accumulation in *Eucalyptus camaldulensis* seedlings irrigated with mixed industrial effluents. Bioresource Technology **88**: 221-228.

Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**: 248-254.

Dey, P.M. (1990) Methods in Plant Biochemistry, Vol-II. Carbohydrates. (Publ.) Academic Press London.

Devi, S.R. and Rani, G. (2017) A study of concentration of sugar mill effluents on properties of soil and types of microorganisms present in the soil. Current botany **8**: 159-162.

Ericson, M.C. and Alfinito, A.E. (1984) Proteins produced during salt stress in tobacco cell cultures. Plant Physiology **74**: 506-509.

Heath, R.L. and Packer, L. (1968) Photo peroxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives in Biochemistry andBiophysics* **125:** 189-198.

Hiscox, J.D. and Israelstam, G.F. (1979) A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian journal of botany* **57**:1332-1334.

Hussain, I., Iqbal, M., Nawaz, M., Rasheed, R., Perveen, A., Mahmood, S., Yasmeen, A. and Wahid, A. (2013). Effect of sugar mill effluent on growth and antioxidative potential of maize seedling. *International journal of Agriculture and Biology* **15**: 1227-1235.

Kaushik, P., Garg, V.K. and Singh, B. (2005) Effect of textile effluents on growth performance of wheat cultivars. Bioresource Technology **96**: 1189-1193.

Kumar, V. and Chopra, A.K. (2014) Pearl millet (Pennisetum Glaucum L.) response after ferti-irrigation with sugar mill effluent in two seasons. *International journal of Recyclingof Organic Waste Agriculture* **3**(67): 1-14.

Mane, P.C., Kadam, D.D., Chaudhary, R.D., Papade, S.E., Waghule, K.K. and Gaikwad, P.A. (2015) Comparative study of untreated and bioremediated sugar industry effluent for irrigation with reference to biochemical attributes of *Triticum aestivum*. *European journal of experimental biology* **5**:46-51.

Olorunfemi, D.I. and Lolodi, O. (2011) Effect of cassava processing effluents on antioxidant enzyme activities in *Allium cepa* L. Biokemistri **23**(2): 49-61.

Pedreroa, F., Kalavrouziotis, I., Alarcóna J.J., Koukoulakis, P. and Asanoc T. (2019). Use of treated municipal wastewater in irrigated agriculture—Review of some practices in Spain and Greece. Agricultural Water Management **97**: 1233–1241

Sarma, H. and Sarma, C.M. (2007) Impact of the fertilizer industry effluent on plant chlorophyll, proteins and total sugars. Nature Environment and Pollution Technology **6**(4): 633-636.

Selvaraj, K., Sevugaperumal, R. and Ramasubramanian, V. (2012). Nullifying the toxicity of plate making industry effluent using seaweed *Ulva lactuca*. *Indian journal of Fundamental and appllied life sciences* **2**(2): 79 -86.

Srivastava, S., Chopra, A.K. and Pathak C. (2012). Ferti-irrigational impact of distillery effluent and Di-ammonium phosphate on the soil and growth characteristics of Eggplant (Solanum melongena L.) *Journal of Applied and Natural science* **2**: 275-283.

Vaithiyanathan, T. and Sundaramoorthy, P. (2017). Analysis of sugar mill effluent and its influence on germination and growth of African marigold (*Tageteserecta* L.) Applied Water **7**:4715–4723.