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# Soil irrigation effect of sugar mill effluent on changes of growth and biochemical contents of *Raphanus sativus* L.

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Abstract
The effect of sugar mill effluent on plant growth and biochemical constituents of *Raphanus sativus* L. var. Pusha Chetki was studied in a pot culture experiment. The experiment was conducted at Botanical Garden, Department of Botany, Annamalai University, Tamil Nadu, during the period of January to March 2008. In the pot culture experiment, radish plants were grown up to 60 days, in the soil irrigated with different concentrations of sugar mill effluent (viz, 0, 20%, 40%, 60%, 80% & 100%v/v). The inner surface of pots was lined with a polythene sheet. Each pot containing 5kg of air dried soil. Six seeds were sown in each pot. All pots were irrigated (500ml) with respective concentration of test solutions daily. Plants were thinned to a maximum of three per pots, after a week of germination. The higher sugar mill effluent concentrations (above 40%) were found to affect plant growth and decreased chlorophyll-a, chlorophyll-b and total chlorophyll, caroteinoids, total sugar, amino acids and protein contents, but diluted effluent (up to 40% ) favoured the plant growth and biochemical contents.

Keywords: sugar mill effluents, radish, growth, and biochemical contents.

## INTRODUCTION

Utility potential of industrial effluents for irrigation of crop-fields has been a controversial proposition due to the contradictory reports obtained on the effects of these effluents on crop plant responses (Sutton et al., 1978; Ajmal et al., 1984; Fayez and Shahin, 1987). Most of our water resources are gradually becoming polluted by addition of huge amounts of sewage, and industrial effluents. These effluents contain toxic materials with varying properties from simple nutrients to highly toxic substances. The discharge of industrial effluents with varying amounts of pollutants has altered the water quality. The sugar industry is playing an important role in the economic development of the Indian sub continent, but the effluents released produce a high degree of organic pollution in both aquatic and terrestrial ecosystems. They also alter the physico-chemical characteristics of the receiving aquatic bodies and affect aquatic flora and fauna. Sugar factory effluent, when discharged into the environment, poses a serious health hazard to the rural and semi-urban populations that uses stream and river water for agriculture and domestic purposes, with reports of fish mortality and damage to the paddy crops in these areas due to wastewaters entering agricultural land (Baruah et al. 1993). Sugar factory effluent has an obnoxious odour and unpleasant colour when released into the environment without proper treatment. Farmers have been using these effluents for irrigation, and found that the growth, yield and soil health was reduced. The effects of various industrial effluents on seed germination, growth and yield of crop plants have captivated the attention of many workers (Ozoh and Oladimeji, 1984; Rahman et al. 2002; Street et al. 2007). However, no detailed experiments have been performed on the plant growth and biochemical changes using sugar factory effluent. In the present investigation, an attempt has been made to study the effects of sugar factory effluent on the growth, chlorophyll, carotenoid, total sugar, amino acids and proteins contents of radish plants.

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## MATERIALS AND METHODS

### Seed materials

The certified seeds of *Raphanus sativus* (L.) cultivar, Pusa Chetki were purchased from Tamil Nadu Agricultural University, Agricultural Research Station, Paramakudi, Ramanathapuram district. Seeds with uniform size, colour and weight were chosen for the experimental purpose.

### Experimental soil

The soil used in the experiment was sandy loam in nature and the pH of the soil was 7.1. It contains 118 kg available N, 28.8 kg available P and 10.9 kg available K/ha, and micro nutrients of 21.89mg available Cu, 219.11mg Fe, 168mg Mn and 28.13mg Zn/kg.

### Collection of sugar mill effluents

The effluent samples were collected in plastic container from the point of disposal from a sugar factory locate in Cuddalore District, Tamil Nadu, India, and stored in cold room until used physico-chemical parameters, such as temperature, colour, pH, biological oxygen demand (BOD), total solids (TS), total suspended solids (TSS), total dissolved solids (TDS), chloride, alkalinity, total hardness, calcium, magnesium, sulphate, phosphate and total iron, were measured using standard methods (APHA,1998). The different concentrations of the effluent (20%, 40%, 60%, 80% and 100%) were prepared and used for the pot culture experiment.

## Pot culture experiment

The experiment was conducted at Botanical Garden, Department of Botany, Annamalai University, Tamil Nadu, during the period of January to March-2008. The impact of sugar factory effluent on the growth and biochemical characteristics of the radish was first investigated using soil pots (15 cm height 15 cm width). About 3kg of air dried soil taken into separate pots. Five different concentrations (viz., 20%, 40%, 60%, 80% and 100%) of sugar mill effluent were prepared and poured into each pot. The control was also maintained and irrigated with tap water. The inner surfaces of pots were lined with a polythene sheet. Six seeds pre-sterilized with 0.1% mercuric chloride, were sown in each pot. All pots were irrigated with 500ml of respective concentration of test solutions daily. Plants were thinned to a maximum of three per pots, after a week of germination. Each treatment including the control was replicated five times. Data points in the tables and figures represent the means, with all deviation bars shown (±1 standard deviations of mean). Both the mean and standard deviation were performed where appropriate using the statistical package on Microsoft_ Excel Version 2003.

## Growth analysis

The plant samples were collected on 60th days after sowing. Three plants from each replicates of pot was analysed for the various growth parameters such as length of root and shoot, number of leaves and leaf area was calculated by measuring the length and width and multiplied by a correlation factor (0.69), derived from the method of Kalra and Dhiman (1977).

## Biochemical estimations

Leaves of treated and control plants were used for the estimation of total chlorophyll as per Arnon (1949), carotenoids as per Kirk and Allen (1965), total sugar as per Nelson (1944), amino acids as per Moore and Stein (1948) and protein contents as per Lowry et al. (1951) methods.

## RESULTS AND DISCUSSION

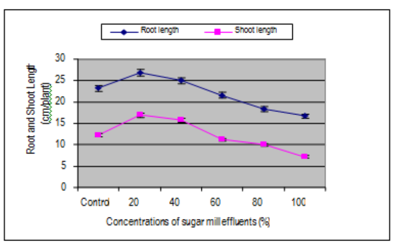
The physico-chemical parameters of the effluent were found to be above those permissible by the Indian Standards (Table-1). The analysis of sugar mill effluent showed that it is acidic in nature with brown in colour. It contained high amounts of suspended and dissolved solids. It showed a high value of Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD). The presence of considerable amounts of calcium, magnesium, chloride, sulphate, fluoride, nitrate and silica, were also noticed in the effluent. This is in conformity with the earlier findings of Chandrasekar et al. (1998), Rathore et al. (2000) and Borole and Patil (2004).

Table 1 Physico chemical parameters of sugar factory effluent

| Parameters | Value |
|---|---|
| Colour | Brown |
| Odour | Decaying smells |
| Temperature | 36 |
| Suspended solids | 520 |
| Ph | 6.1 |
| EC (µS cm-1) | 1.0 |
| DO | 10.1 |
| BOD | 2980.0 |
| COD | 4947.0 |
| TS | 1344.0 |
| TDS | 2398.0 |
| Chloride | 850.0 |
| Alkalinity | 120.0 |
| Hardness | 900.0 |
| Calcium | 430.0 |
| Magnesium | 540.0 |
| Sulphate | 388.95 |
| Phosphate | 23.0 |
| Iron | 0.08 |
| Oil and Greece | 19.0 |

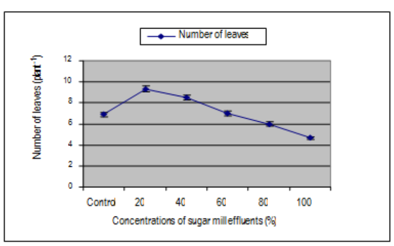
## Growth

Root and shoot length of radish plants differed with different concentrations of sugar mill effluents in the soil (Fig-1). For lower concentrations of irrigated effluent (20% and 40%), the root and shoot length of radish plants were higher than that of control plants, which may be taken as an indication of beneficial range, while for higher concentrations of effluents (60%,80% and 100%) a decreasing trend was observed, which confirms the toxic effect of this effluents to radish plants. The above results were in agreement with the findings of Kaushik et al. (2004) who reported a clear toxicity of sugar factory effluent on the growth, photosynthetic pigments and nutrient uptake in wheat seedlings in aqueous versus soil medium. The presence of calcium and magnesium cause higher osmotic pressure, resulting in the wilting of seedlings (Gomathi and Oblisami 1992). In our study, the plant growth was highly affected due to the excess amount of chloride, alkalinity, hardness, calcium, magnesium, sulphate and phosphate in the sugar factory effluent. The root length was severely affected by the higher effluent concentrations (100%) for radish (16.2cm) compared to the control. Similarly, radish showed maximum number of leaf (9.50, 8.66) and leaf area (96.22, 84.00), in the 20% and 40% effluent concentrations and these values were minimum in 100% effluent (Fig-2 and 3) treatment.



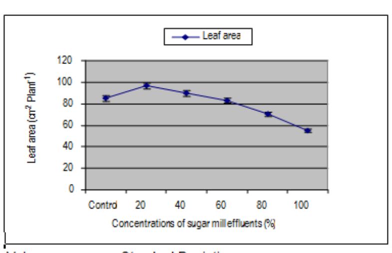

Values are mean ±Standard Deviations

Figure-1 Effect of sugar mill effluents on root and shoot length of radish plants



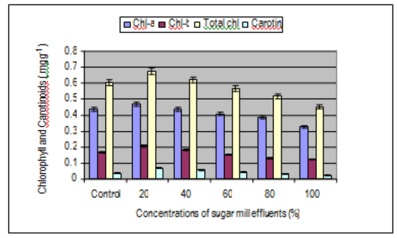

Values are mean ±Standard Deviations

Figure-2 Effect of sugar mill effluents on leaf number of radish plants.



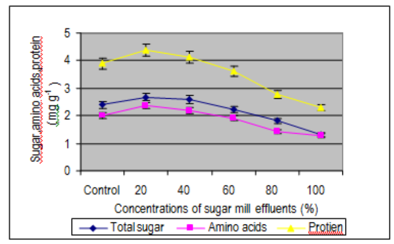

Values are mean ±Standard Deviations

Figure-3 Effect of sugar mill effluents on leaf area of radish plants.




Values are mean ±Standard deviations

Figure-4 Effect of sugar mill effluents on chlorophyll-a, chlorophyll-b, total chlorophyll and carotinoids contents of radish plants.




Values are mean ±Standard deviations

Figure-5 Effect of sugar mill effluents on total sugar, amino acids and protein contents of radish plants

## Biochemical estimations

Maximum photosynthetic pigments such as chlorophyll a, chlorophyll b total chlorophyll and carotenoid of radish were observed at 20% and 40% concentrations of sugar mill effluent in the soil. The increased chlorophyll content was obviously due to sugar mill effluent at low concentrations which act as structural and catalytic components of proteins, enzymes and as cofactors for normal development of chlorophyll biosynthesis. In higher concentrations, sugar mill effluent (60%, 80% and 100%) become toxic to plants and a decrease in photosynthetic pigments was observed (Fig-4). Our results are in consonance with the findings of Nagajyothi et.al. (2008) who reported that the increased chlorophyll content at lower concentration may be due to the favorable elements present in the effluent on the pigment system. Iron, magnesium, potassium, zinc and copper are essential for the synthesis of chlorophyll was proposed by Swaminathan and Vaidheeswaran, (1991). The increase in the chlorophyll, carotenoids content of plants at lower concentration of the effluent might be due to the favorable effect of nitrogen and other inorganic elements which are present in their optimum quantities. It may be due to the decrease in the chemical concentrations to an optimum level on the dilution of the effluent. The increase in carotenoid content might be due to enhanced influence of nitrogen and other organic elements present in the effluent Subramani, et al. (1999). Presence of magnesium and potassium in their optimum quantities in the lower concentration of the effluent which are required for biosynthesis of pigment. In the chlorophyll estimation, chlorophyll a, b, total chlorophyll and carotenoids content of the plant significantly affected under sugar factory effluent irrigation. Reduction in chlorophyll content induced by effluent may be associated with mineral ions. Some of the possible reasons for the decrease in chlorophyll content may be the formation of enzyme such as chlorophyllase which is responsible for chlorophyll degradation (Gupta et al. 2004; Saravanamoorthy and Ranjitha Kumari, 2005 and Nagajyothi et al. 2008)

Total sugar contents showed a decreasing trend with progressive increase in sugar mill effluent concentration in radish. However, 20% and 40% effluent concentrations produced positive effect on the sugar contents (Fig-5). The same trend was recorded with treatment implies the deranged starch metabolism and poor translocation of sugar to growing parts by Chandrasekar et al. (1998) and Subramani et al. (1999). The decrease in sugar content may be due to the increase in the concentration of various cations and anions present in the effluent. These results are in conformity with the results of Chandrasekar et al. (1998) and Swaminathan and Vaidheeswaran (1991). The increase in the amount of sugar might be either due to inhibition in starch synthesis from hexose or stimulation of starch hydrolysis was recorded by Murata et al. (1969).

Amino acid and protein content of radish were higher at low (20% and 40%) concentration of sugar mill effluent in the soil than in the control plants. Further, the values decreased with a gradual increase in effluent (60%, 80% and 100%) concentration. (Fig-5). Several authors contributed various reasons for the reduced amounts of amino acid and protein contents due to sugar mill effluent. Plants treated with higher effluent concentrations (above 40%) showed lower amounts of amino acid and protein contents due to the presence of higher magnesium concentrations and the acidic pH of the effluent. Calcium and magnesium (20 mg/L) influence plant growth, biomass partitioning and fruit yield, and create symptoms of leaf chlorosis after 8 weeks in green house tomato (Hao and Athanasios, 2004). Lasa et al. (2000) also reported that four different concentrations (0.1, 0.8, 5 and 10 mM) of magnesium affected the growth of sunflower plants grown with ammonium and nitrate and they also proved that the magnesium- fed plants had lower free amino acids and soluble protein contents in their leaves. Decrease in free amino acids at high salinity concentrations can be attributed to the inhibitory effect of the effluent on protease activity (Pulver and Ries, 1973 and Joshi and Tandom, 2003). The significant increase in the protein content of plant might be due to the potassium and nitrate in their optimum quantity present in the lower concentration of the effluent as reported by (Kadioglu and Algur, 1990) in pea plants.

## CONCLUSION

This study concluded that the physico-chemical parameters, such as BOD, chloride, alkalinity, hardness, calcium, magnesium, sulphate and phosphate were relatively higher in the sugar factory effluent and severely affected the plant growth. There was a gradual decrease in the root and shoot length, number of leaves, leaf area, and chlorophyll a, b, total chlorophyll and carotenoids, total sugar, amino acid and protein contents in radish plants, when irrigated with various effluent concentrations (except 20% and 40%) compared to the control. Effluent at 20% and 40% concentration favoured the plant growth and increased the growth and biochemical contents. This may be attributed to the optimum levels of inorganic nutrients and reduction in toxicity level due to dilution. Thus the effluent after diluting up to 40% can be used for irrigation as soil fertilizers for the better growth, biochemical and yield of radish. In the beneficial effluent concentrations, 20% was better than 40%.

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