Journal of Environmental Treatment Techniques 2016, Volume 4, Issue 2, Pages: 52-57

Journal of ENVIRONMENTAL Treatment Techniques

J. Environ. Treat. Tech.
ISSN: 2309-1185

Journal weblink: http://www.jett.dormaj.com

Sugar Mill Effluent Induced Changes in Germination and Biochemical of Hybrid Brinjal (*Solanummelongena* l. var. pruthvi)

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Received: 29/01/2015 Accepted: 14/09/2015 Published: 30/06/2016

Abstract

Sugar mills play a major role in polluting the environment specially water bodies and land by discharging their effluent. This polluted water is being used for irrigation due to water scarcity. It harmfully affects the growth and yield of crops. In the present study, physico-chemical parameters of sugar mill effluent were recorded. It not only contains the toxic substances but also having same amount of nutrients which are needed for the growth of plants. The effect of various concentrations (control, 10, 25, 50, 75 and 100%) of sugar mill effluent on seed germination behaviour of hybrid brinjal (*Solanum melongena* L. var. Pruthvi) was studied. The morphological growth parameters such as germination percentage, seedling length, seedling fresh weight, seedling dry weight, vigour index, and phytotoxicity were taken into consideration. The result shows that the lower concentration (10 percent) of effluent increased the germinating percentage and growth and higher concentrations (25, 50, 75 and 100%) of effluent decreased the germination percentage, seedling growth, fresh weight and dry weight of brinjal and also biochemical such as chlorophyll, amino acid and starch.

Keywords: *Solanum melongena L.*, sugar mill effluent, seed germination, phytotoxicity

1 Introduction

India is an agricultural country and a major user of water resource for irrigation. The scarcity of water is a major problem in the arid and semi-arid regions of India. The use of the industrial waste water for agricultural irrigation has emerged in the recent past as an alternative source of water in these areas. Pollution is an undesirable change in physical, chemical and biological aspects of our environment. Among all kinds of pollution the problems of water pollution due to the industrial effluent as well as sewage wastes have attained the grater dimensions day by day in India [1]. Water pollution may be defined as natural or induced changes in the quality of water which renders it unusable or dangerous as regard to food, human and animal health, industry, agriculture and fishing [2].

The most important effluent discharging industries are thermal power plants, paper mills, textiles, distilleries, fertilizer units, electroplating ,detergents, iron and steel industries, pharmaceuticals, petrochemical, pesticide and herbicides, oil refineries , tannery and sugar mill industries. The industries are classified into three categories such as red (high polluting industry), orange (less polluting industry) and green (non-polluting industry) by Ministry of Environment and Forests, New Delhi on the basis of the potentiality in creating pollution. In our country, there are more than 20 types of industries coming under the red category, which are highly polluting the environment. Indiscriminately discharged industrial effluents contain organic and inorganic compounds, suspended solids and other materials. The disposal of industrial effluent into land or water bodies without prior treatment is a common practice in India. The danger associated with heavy metals, toxic ions present in untreated effluent and sewage have become great social and scientific threats.

Sugar industries play a major role in creating the polluted environment. It falls under the red category. It plays a major role in rural economy of our country. At the same time, it caused a considerable amount of water and soil pollution by releasing a large amount of waste water from the sugar mill. The effluent is discharged into nearby water bodies making them polluted ones. The polluted water is being used for irrigation by nearby farmers. Polluted water irrigation was directly affect soil but also reduced agricultural yield [3]. The present research work has been made to assess the physic-chemical characteristics of sugar mill effluent and its effects on germination behaviour and seedling growth of hybrid brinjal (*Solanum melongena* L. var. Pruthvi)

2 Materials and Methods

Effluent samples

The effluent samples were collected in plastic containers from the out let of the N.P.K.R. Co-operative sugar mill in Thalainayar, Mayiladuthurai Taluk, Tamil Nadu and India. They were brought to the Ecology Laboratory, Department of Botany and stored in refrigerator at 4ºC for analysis purpose.

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Seed material

Hybrid brinjal (*Solanum melongena* L. var. Pruthvi) seeds were procured from Authorized Private Agro Centre, Chidambaram, Cuddalore district, Tamil Nadu.

Analysis of sugar mill effluent sample

The collected sugar mill effluent sample was analysed for their various physico-chemical properties in Ecology Laboratory, Department of Botany, Annamalai University as per the routine standard methods mentioned in American Public Health Association [4].

Preparation of different concentrations of effluent

The collected effluent sample from the outlet of sugar mill industry was treated as 100 per cent raw effluent. Different concentrations (10, 25, 50, 75 and 100%) of sugar mill effluent were prepared freshly by using distilled water whenever necessary. They were used for germination studies.

Control	:	Distilled water
10%	:	10 ml effluent + 90 ml water
25%	:	25 ml effluent + 75 ml water
50%	:	50 ml effluent + 50 ml water
75%	:	75 ml effluent + 25 ml water
100%	:	Raw effluent

Germination studies

The healthy and uniform sized hybrid brinjal seeds were selected and surface sterilized with 0.1% $HgCl_2$ for two minutes. They are thoroughly washed with tap water to avoid surface contamination. Twenty seeds were placed equidistantly in petridishes filled with sterilized soil. The seeds were irrigated with equal quantity of different concentrations of effluent and the seeds irrigated with distilled water treated as control. Five replicates were maintained for each treatment including control. The germination percentage, seedling length, seedling fresh weight and seedling dry weight were taken and recorded on the 21^{st} day's seedlings. The values of vigour index and percentage of phytotoxicity were also calculated. At the same time photosynthetic pigments (chlorophyll a, chlorophyll b and total chlorophyll) and other biochemical analyses such as amino acids and starch were also analyzed.

Germination percentage

The number of seeds germinated in each concentration was counted on the 21^{st} day and the germination percentage was calculated by using the following formula:

$$GP = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Shoot and root length (cm/seedling)

Five seedlings were taken from each treatment and their shoot length and root length were measured by using a cm scale and these values were recorded.

Fresh weight (g/seedling)

Five seedlings were collected from each treatment and their fresh weights were measured with the help of an electrical single pan balance.

Dry weight (mg/seedling)

The same seedlings used for fresh weight were kept in hot air oven at 80°C for 24 hours. Then, the seedlings were taken from the oven and kept in desiccators for some time. Their dry weights were taken by using an electrical single pan balance.

Vigour index

Vigour index of the seedlings was calculated by using the [5].Vigour index = Germination percentage × Length of seedling

Percentage of phytotoxicity

The percentage of phytotoxicity of effluent was calculated by using the formula [6].

Percentage of phytotoxicity =

$$\frac{\text{Radicle length of control - Radicle lenght of test}}{\text{Radicle length of control}} \times 100$$

Biochemical analysis

Estimation of chlorophyll [7]

0.5 g of fresh leaf material was taken and ground with 10 ml of 80 per cent acetone in pestle and mortar. The homogenate was centrifuged at 800 rpm for 15 minutes. The supernatant was saved. The residue was reextracted with 80 per cent acetone. The supernatant was saved and utilized for chlorophyll estimation. Absorbance was read at 645, 663 and 480 nm in Spectrophotometer.

Total chlorophyll (mg g^{-1}fr. wt.) = (0.0202) × (OD 645) + (0.00802) × (OD663)
Chlorophyll 'a' (mg g^{-1}fr. wt.) = (0.0127) × (OD 663) – (0.00269) × (OD 645)
Chlorophyll 'b' (mg g^{-1}fr. wt.) = (0.229) × (OD 645) – (0.00488) × (OD 663)

Carotenoid (mg/g fr. wt.)

Carotenoid content can be calculated (8).
Carotenoid = (OD 480) – (0.114) × (OD 663) – (0.638) × (OD 645)

Estimation of amino acid [9]

0.5 grams of plant material were ground well with 10 ml of 80 per cent ethanol in a pestle and mortar. The homogenate was centrifuged at 800 rpm for 10 minutes and the supernatant was saved. The supernatant made upto 10 ml with 80 per cent ethanol. 1 ml of extract was taken and 1 ml of ninhydrin reagent were added and mixed thoroughly in a Folin-Wu tube and the content was heated for 20 minutes in a boiling water bath at 100°C. After 20 minutes, the content was removed from the water bath and cooled under tap running water. The content was mixed thoroughly made upto 10 ml with diluting solution. Then, the solution was read at 570 nm in a Spectrophotometer.

Starch content [10]

The ethanol insoluble residue left behind after alcoholic extraction of the original material was taken for the extraction of starch.The residue was dissolved in 6.5 ml of 52 per cent perchloric acid for one hour. It was centrifuged and made upto 100 ml in a volumetric flask with distilled water. One ml of this solution was further diluted with 5 ml of distilled water. To this, 10 ml freshly prepared anthrone reagent was added. The content was heated for 7 minutes at 100ºC in a boiling water bath. The tube was then cooled rapidly, shaken well and the appeared colour was read at 630 nm in Spectronic – 20.

3 Results and Discussion

Physico-chemical properties of sugar mill effluent are given in Table – 1. The analyses of sugar mill effluent showed that it is acidic in nature with dull white in colour. It contained high amounts of suspended solids 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, chloride, sulphate, nitrate, fluoride, and silica were also noticed in the effluent. This is in conformity with the earlier findings [11, 12, 13, 14, 15, 16, 17, 18]. The pollution load of the effluent depends upon the nature of raw materials, chemicals used, the processes involved in the factory and also the methods of treatments given to the effluent before they discharged from the factory.

Table 1: Physico – Chemical properties of sugar mill effluent

S. No.	Properties	Raw effluent
1	Colour	Dull white
2	Odour	Decaying molasses smell
3	pH	4.04
4	Electrical conductivity (EC)	4745 Mm- homs
5	Temperature (^{0}C)	33.0
6	Acidity	1350.0
7	Suspended solids	180.0
8	Total dissolved solids	3725.0
9	Total solids	3905.0
10	BOD	3480.0
11	COD	7880.0
12	Chloride	314.0
13	Sulphate	290.88
14	Nitrate	57.59
15	Fluoride	1.88
16	Silica	99.0
17	Calcium	124.8

All parameters except colour, odour, pH, EC and temperature are expressed in mg/l.

The effect of different concentrations of sugar mill effluent on germination studies of hybrid brinjal is presented in the above the figure -1. In the present investigation, the higher concentration (25, 50, 75 and 100%) of sugar mill effluent did not help plant germination because the presence of high salt content in the effluent at these concentrations. Seed absorption water during germination and hydrolyse stored food material and to activate enzymatic systems. During germination salts can inhibit germination. The high salt concentration can slow germination by several days or completely inhibit it [19]. The lower concentration of sugar mill effluent (10%) promoted the germination. It may be due to presence of optimum level of nutrients in this effluent concentration. Similar observation was recorded in soybean [20, 21, 22].

Seedling growth and their fresh and dry weight of hybrid brinjal seedlings increased at lower concentration o f sugar mill effluent. It may be due to maximum uptake of nitrogen, phosphorus and potassium by plants from this effluent concentration. The improvement of vegetative growth may be ascribed to the role of potassium in nutrient and sugar translocation in plants and turgor pressure in plant cells [23]. However, the higher concentrations of sugar mill effluent decreased in above the vegetative parameters of the brinjal plants. In higher concentrations of sugar mill effluent contains high amount of organic and inorganic compounds, it becomes toxic to plants. Similar observations were obtained by several workers [24, 25, 26].

The above figure -2 denote the maximum photosynthetic pigments such as chlorophyll a, chlorophyll b and total chlorophyll of brinjal were observed at 10% concentrations of sugar mill effluent may be due to the favourable elements such as magnesium, potassium etc., present in the effluent on the pigment system. In higher concentrations of sugar mill effluent become toxic to plants and a decrease in photosynthetic pigments were observed above the figure -2. The findings were also recorded by many researchers at different crops [27, 28, 29]. The increase in the amino acids and starch content of plants at lower concentration of the effluent might be due to the favourable effects of organic and inorganic elements which are present in their required quantities. But, the higher concentrations of sugar mill effluent decreased the above mentioned parameters. These results are in conformity with the results of some researchers [30, 31, 32]. The 10% sugar mill effluent contains optimum content of nutrients required for maximum vegetative growth of brinjal plant. While studied the effect of various industrial effluents on germination studies of some agricultural crops. The increase in germination study parameters at lower concentration may be due to presence of growth promoting nature of nutrients in the diluted effluent. The reductions in germination percentage and seedling growth at higher concentrations of effluent are due to the presence of excess amount of minerals present in the effluent. These excess amount of minerals inhibit the germination and growth by interfering the metabolic activities during germination and growth [33, 34].

4 Conclusions

The physico-chemical parameters such as TDS, BOD and COD were observed to be higher in sugar mill effluent and it severely affected the plant growth. From this study, results that the lower concentration of sugar mill effluent (10%) promotes the seed germination percentage, vegetative growth and biochemical of hybrid brinjal seedlings and their higher concentrations of sugar mill effluent inhibited the seed germination percentage, vegetative growth and biochemical of hybrid brinjal seedlings compared to the control. It can also be concluded that not only toxic metals but higher nutrients can also be toxic and inhibits the seed germination and seedling growth. Therefore, dilution of the sugar mill effluent is necessary to minimize the toxicity of sugar mill effluent and it has the potentiality as organic fertilizer. However, the lower concentration of effluent can be utilized for agricultural irrigation after suitable treatment with appropriate dilution.

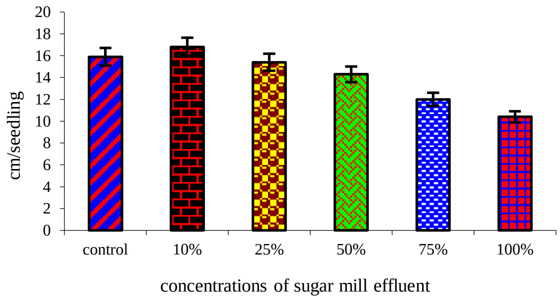

Figure 1.2: Seedling length

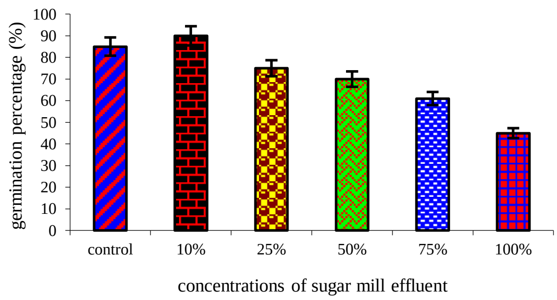

Figure 1.1: Germination percentage

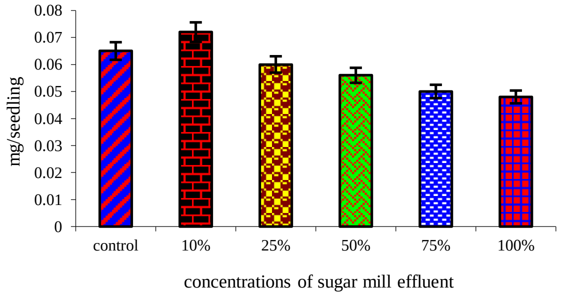

Figure 1.4: Dry weight

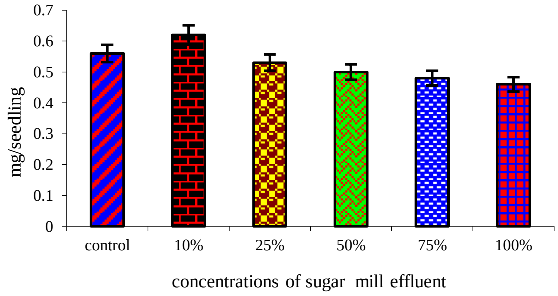

Figure 1.3: Fresh weight

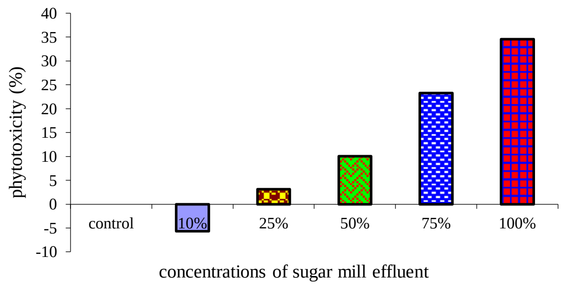

Figure 1.6: Percentagy of phytotoxicity

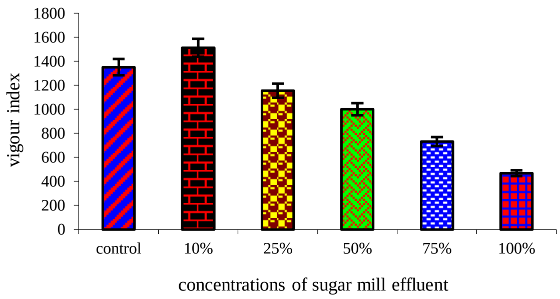

Figure 1.5: Seed vigour index

Figure 1: Germination studies of Hybrid Brinjal grown under different concentrations of the Sugar mill effluent.

Plate 1: Shows on 21st DAS of Hybrid Brinjal grown under different concentrations of the Sugar mill effluent.

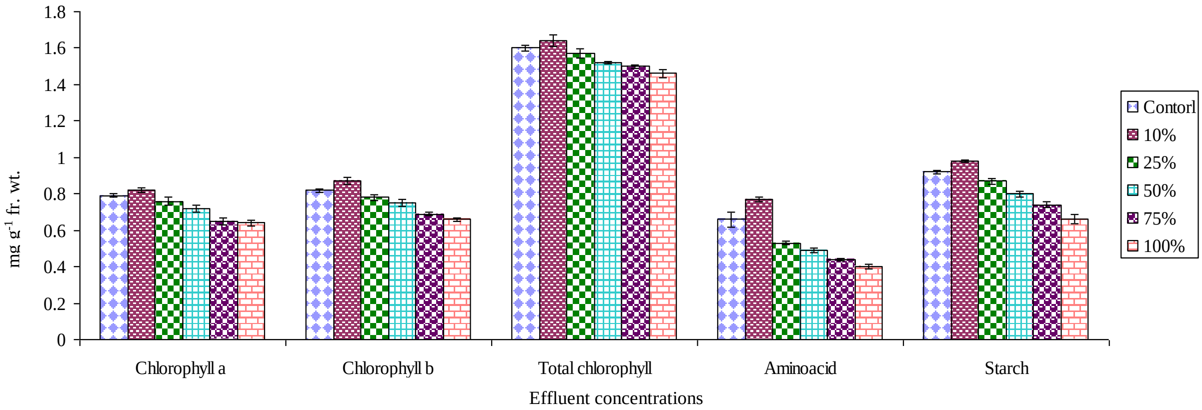

Figure 2: Biochemical analyses of Hybrid Brinjal grown under different concentrations of the Sugar mill effluent.

Acknowledgement

The authors are thankful to Professor and Head, Department of Botany, Annamalai University for providing laboratory facilities to carry out the research experiments.

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