Growth and Physiological Activity of Greengram
(Vigna radiata L.) Under Effluent Stress

L. Baskaran, P. Sundaramoorthy, A.L.A. Chidambaram and K. Sankar Ganesh

Abstract: The present research work has been carried out to understand the effect of different concentrations of sugar mill effluent on growth, yield, biochemical contents and enzymatic activities of greengram (Vigna radiata (L.) Wilczek). The increasing pace of industrialization in public and private sectors along with urbanization, population explosion and green revolution are reflected in varying degree of pollution of water, soil and air. The sugar mill effluent is having a higher amount of organic and inorganic elements. The physico-chemical analysis showed that it was acidic in nature and yellowish in colour. It was rich in total suspended and dissolved solids with large amount of Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD). The higher amount of chloride, calcium, magnesium, sodium, potassium and iron were also present in the effluent. The effluents severally affect crop plants and soil properties when used for irrigation. Pot culture experiments were conducted with greengram at different concentrations of sugar mill effluent. The growth parameters such as germination percentage, shoot length, root length, Fresh Weight (FW), Dry weight (DW) and leaf area were measured at 15, 30, 45 and 60 Days After Sowing (DAS). The pigment analyses viz. chlorophyll ‘a’, chlorophyll ‘b’, total chlorophyll, protein, amino acid, sugar contents and enzymes activities were analysed at 15 and 60 DAS. The yield parameters of greengram plants were recorded at the time of harvest. All morphological growth parameters, biochemical contents, enzyme activities and yield parameters were found to increase at 10% effluent concentration and it decreased from 25% effluent concentration onwards. So, these results reflect that the sugar mill effluent is toxic to crop and it can be used for irrigation purpose after a proper treatment with appropriate dilutions.

Key words: Sugar mill effluent • Vigna radiata • Growth • Biochemicals • Antioxidant enzymes • Yield

INTRODUCTION

Industrialization is an important tool for the development of any nation. Consequently, the industrial activity has expanded so much all over the world. Today, it has become a matter of major concern in the deterioration of the environment [1]. With the rapid growth of industries (sugar, paper, tannery, textile, sago, dye industries) in the country, pollution of natural water by industrial waste water has increased tremendously [2]. Among them, sugar industry plays a major role in producing a higher amount of water pollution because they contain large quantities of chemical elements. They contain higher amounts of total hardness, total dissolved solids, biological oxygen demand, chemical oxygen demand, calcium, magnesium, sodium, iron and sulphate. The effluent not only affect the plant growth but also deteriorate the soil properties when used for irrigation [3]. In addition to that, some traceable amount of heavy metals such as zinc, copper and lead were also present in the effluent [4]. In arid and semi-arid regions of our country where shortage of water becomes limiting factor in agriculture, the effluent mixed polluted water is used for irrigational purposes by farmers [5]. As this polluted water is being used for irrigation to cultivate the crops, it is necessary to conduct experiments to find out the impact of these effluents on agricultural crops before they are recommended for irrigation. The study on the impact of sugar mill effluent on germination of crops has been already made [6,7]. The research work on growth and yield of crop plants is very rare So, the present investigation deals with the impact of sugar mill effluent irrigation on germination, growth, biochemical contents and enzyme activities and yield of greengram (Vigna radiata (L.) Wilczek).

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MATERIALS AND METHODS

Collection of Effluent and Seed Materials: Sugar mill effluent samples were collected from the point of discharge of sugar factory situated in Tamil Nadu, India. The effluent sample was collected in plastic containers and stored in refrigerator. The effluent was analyzed for its various physico-chemical parameters as per the method of APHA [8]. The seeds of green gram (*Vigna radiata* (L.) Wilczek) were procured from Department of Pulses, School of Genetics, Tamil Nadu Agricultural University, Coimbatore, India. The surface sterilized green gram seeds were equispacially arranged in earthen pots containing five kg of garden soil. The various concentrations (10, 25, 50, 75 and 100%) of sugar mill effluent solution were prepared and used for both laboratory and pot culture studies. The pots were irrigated with equal volumes of various concentrations of sugar mill effluent. Control set was irrigated with equal volume of bore-well water. The morphological parameters, biochemical contents and enzyme activities were analyzed at 15 days intervals. Five plants were collected from each treatment and they were analysed for their morphological parameters such as shoot length, root length, total leaf area, fresh weight and dry weight of the crop plant. The number of seeds germinated in each concentration was counted on 7th day and the germination percentage was calculated by using the following formula:

\[
\text{Germination Percentage} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds sown}} \times 100
\]

Fresh weight (g/plant) were taken with the help of an electrical single pan balance. The collected plant materials were kept in hot air oven at 80°C for 24 hours and their dry weight (g/plant) were taken by using an electrical single pan balance. The leaf area was calculated by measuring the length and breadth of the leaf was described by Yoshida [9].

\[
\text{Leaf area (cm}^2\text{)} = K \times \text{length} \times \text{breadth}
\]

Where, \(K\) = Kemp’s constant (for dicot leaves = 0.66). Plant samples were taken at the time of harvest for the observation of the yield parameters such as number of pods plant\(^{-1}\), number of seeds pod\(^{-1}\) and 100 seed weight.

Estimation of Chlorophyll Arnon [10]: Five hundred mg of fresh leaf material was taken and ground with help of pestle and mortar with 10 ml of 80% acetone. The homogenate was centrifuged at 800 rpm for 15 minutes. The supernatant was saved. The residue were re-extracted with 80% acetone. The supernatant was saved and utilized for chlorophyll estimation. Absorbance was read at 645, 663 and 480 nm in the UV-spectrophotometer.

\[
\text{Chlorophyll ‘a’ (mg g}^{-1}\text{FW)} = (0.0127) \times (\text{OD 663}) - (0.00269) \times (\text{OD 645})
\]

\[
\text{Chlorophyll ‘b’ (mg g}^{-1}\text{FW)} = 0.229 \times (\text{OD 645}) - (0.00488) \times (\text{OD 663})
\]

\[
\text{Total chlorophyll(mg g}^{-1}\text{FW)} = (0.0202) \times (\text{OD 645}) + (0.00802) \times (\text{OD 663})
\]

Estimation of Protein: Protein content was determined by the method of Lowery et al. [11]. 0.5 g of plant sample (shoot) was homogenized in 10 ml of 20% Trichloro Acetic Acid (TCA). The homogenate was centrifuged in 10 minutes for 300 g. The supernatant was discharged and the pellet was re-extracted with 5 ml of 0.1 N NaOH. One ml of the extract was taken in a test tube and 5 ml of reagent ‘C’ (protein reagent) was added. This solution was mixed well and kept in dark for 10 minutes. Later, 0.5 ml of folin phenol reagent was added and the mixture was kept in dark for 30 minutes. The sample was read at 660 nm in the UV-spectrophotometer.

Amino Acid: Amino acid content was determined by the method of Moore and Stein [12]. 0.5 g of plant sample was homogenized in 10 ml of 80% ethanol. The homogenate was centrifuged for 10 minutes at 800 g. One ml of the extract was taken in the test tube and add 1 ml of 0.1 N HCl to neutralize the sample. To this, one ml of ninhydrine reagent was added and heated for 20 minutes in a boiling water bath. Later, 5 ml of the diluents solution was added and heated again in water bath for 10 minutes. The test tubes were cooled and read the absorbance at 570 nm in a UV-spectrophotometer.

Estimation of Sugars [13]

Extraction: Five hundred mg of plant materials were weighed and macerated in a pestle and mortar with 10 ml of 80 per cent ethanol. The homogenate was centrifuged for 10 min at 800 rpm. The supernatant was saved. Then, the ethanol is evaporated in a water bath at 50°C. The net content was made upto 20 ml with distilled water and the extract was used for the estimation of reducing sugar.

Estimation: One ml of extract was taken in a 25 ml marked test tube. 1 ml of reagent ‘C’ was added. Then, the mixture was heated for 20 min at 100°C in a boiling water bath, cooled and 1 ml of arsenomolybdate reagent was added.
The solution was thoroughly mixed and diluted to 25 ml with distilled water. The sample was read in the UV-spectrophotometer at 520 nm. The sugar contents were expressed in mg/g fresh weight basis.

**RESULTS AND DISCUSSION**

The physico-chemical properties of sugar mill effluent is given in Table 1. The effluent was yellow in colour with acidic in nature. It contained higher amount of suspended solids, dissolved solids, Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD). In addition, there was a considerable amount of chlorides, sulphates, calcium and magnesium present in the effluent. The same constitutions were also noted in sugar mill effluent by Chandrasekar *et al.* [16]. The effluent contained some heavy metals, such as zinc, copper and lead [4]. The variation in physico-chemical properties of sugar mill effluent may be due to the raw materials and chemicals used in the processes involved in sugar production [17]. The industrial effluents with excess of elements discharged into nearby water bodies and made the early availability of metals to flora and fauna.

Figure 1 shows the germination percentage and the higher germination percentage was observed at 10% sugar mill effluent concentration. At higher concentrations, the germination percentage was gradually decreased from 25 to 100% raw effluent. It may also be due to the disturbance of the osmotic relations of the seed and water, thus reducing the amount of absorbed water and retarding seed germination by enhanced salinity and conductivity of the solutes. Further more, the germinated seeds will not get any oxygen due to organic and inorganic chemicals present in the effluent. Similar findings were also noted by Goel and Kulkarni [18], Chandrasekar *et al.* [16], Priya Kaushik *et al.* [19] and Bishoni and Goutam [20].

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Parameters</th>
<th>Raw effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Colour</td>
<td>Yellow</td>
</tr>
<tr>
<td>2.</td>
<td>Odour</td>
<td>Decaying molasses smell</td>
</tr>
<tr>
<td>3.</td>
<td>pH</td>
<td>4.55-5.25</td>
</tr>
<tr>
<td>4.</td>
<td>Electrical conductivity</td>
<td>2.23</td>
</tr>
<tr>
<td>5.</td>
<td>Temperature (°C)</td>
<td>33.0</td>
</tr>
<tr>
<td>6.</td>
<td>Chloride</td>
<td>26</td>
</tr>
<tr>
<td>7.</td>
<td>Total hardness</td>
<td>245</td>
</tr>
<tr>
<td>8.</td>
<td>Total Dissolved Solids</td>
<td>1480</td>
</tr>
<tr>
<td>9.</td>
<td>Biological Oxygen Demand</td>
<td>1850</td>
</tr>
<tr>
<td>10.</td>
<td>Chemical Oxygen Demand</td>
<td>2359</td>
</tr>
<tr>
<td>11.</td>
<td>Calcium as CaCO₃</td>
<td>91</td>
</tr>
<tr>
<td>12.</td>
<td>Magnesium as Mg</td>
<td>224</td>
</tr>
<tr>
<td>13.</td>
<td>Sodium</td>
<td>40</td>
</tr>
<tr>
<td>14.</td>
<td>Iron</td>
<td>42</td>
</tr>
<tr>
<td>15.</td>
<td>Sulphate</td>
<td>246</td>
</tr>
</tbody>
</table>

All parameters except colour, odour, pH, EC and temperature are expressed in mg l⁻¹.
The increase in germination percentage might be due to the reduction in level of toxic metabolites by dilution and better utilization of nutrients present in the effluent [21]. The reduction in germination percentage at higher concentrations may also be due to the excess amount of minerals and nutrients present in the effluent [22]. Reduction in seed germination percentage at higher concentration of effluent may be due to the higher amount of solids present in the effluent, which causes changes in the osmotic relationship of the seed and water. The reduction in the amount of water absorption take place with results in to reduction of seed germination due to enhanced effluent salinity [23].

In pot culture experiment, the shoot and root lengths were measured at different stages of the growth of greengram and are presented in Fig. 2. The higher shoot and root lengths were recorded at 10% effluent concentration. The root and shoot length were adversely affected by higher concentrations of sugar mill effluent treatment. A gradual decrease in these growth parameters were observed. The same findings were reported earlier due to effluent treatment by Rajesh Kumar and Bhargava [24], Hariom et al. [25]. The reduction in shoot and root growth at higher concentration of effluent may be due to the fact that germinating seeds under higher concentrations would get less amount of oxygen which might have restricted the energy supply and retarded the growth and development [26]. The effluent toxicity was manifested by the hard texture, hypertrophy of seedling, as well as browning and impairment of the root system. The higher toxicity were more pronounced in seedling at higher concentrations effluent [27].

The FW and DW of plant samples grown in various concentrations of effluent were presented in Fig. 3. The FW and DW were also increased at lower concentrations and decreased at the higher concentrations of sugar mill effluent. The presence of optimum level of nutrients in the lower concentrations of sugar mill effluent might have increased the FW and DW of crop plants. The reduction in dry weight of plant materials may be due to the poor growth under effluent irrigation [28].
Table 2: Effect of different concentrations of sugar mill effluent on yield parameters of greengram (Vigna radiata L.) at harvest stage

<table>
<thead>
<tr>
<th>Effluent concentration (%)</th>
<th>Number of fruits plant$^{-1}$</th>
<th>Number of seeds plant$^{-1}$</th>
<th>100 seed weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.2 ± 1.160</td>
<td>127.6 ± 6.38</td>
<td>38.735 ± 1.936</td>
</tr>
<tr>
<td>10</td>
<td>25.0 ± 1.250</td>
<td>146.4 ± 7.32</td>
<td>50.545 ± 2.527</td>
</tr>
<tr>
<td>25</td>
<td>18.5 ± 0.925</td>
<td>120.2 ± 6.01</td>
<td>30.638 ± 1.531</td>
</tr>
<tr>
<td>50</td>
<td>16.2 ± 0.810</td>
<td>108.5 ± 5.43</td>
<td>26.372 ± 1.319</td>
</tr>
<tr>
<td>75</td>
<td>13.0 ± 0.650</td>
<td>102.0 ± 5.10</td>
<td>22.625 ± 1.131</td>
</tr>
<tr>
<td>100</td>
<td>8.5 ± 0.425</td>
<td>65.8 ± 3.29</td>
<td>16.120 ± 0.806</td>
</tr>
</tbody>
</table>

± Standard deviation

Figure 4 shows that there was a significant increase in total leaf area of greengram plants at 10% concentration of sugar mill effluent irrigation. At the same time, leaf areas were reduced in higher concentrations of effluent. It may be due to the reduced cell size and decreased intercellular spaces were largely responsible for reduction in leaf area due to effluent toxicity [29].

The yield parameters such as number of fruits per plant, number of seeds per fruits and their economic yield was higher at 10% effluent concentration (Table 2). The high yield of plants at lower concentrations might depend on the enhanced low biosynthesis of pigments, carbohydrates and proteins [30]. Present observation of increase and decrease in yield parameters also matches with the similar findings of Rajesh Kumar and Bhargava [24]. The findings of the present study reveals that the sugar mill effluent at lower concentrations promotes the growth and yield but the higher concentrations reduced the same though phytotoxic nature of effluent. The effluent invariability contains acids, alkalis, organic, inorganic salts, heavy metals and toxic elements which enter the plant body and disturb indigenous system, often producing detrimental effect [5].

The decrease in shoot length, root length, fresh weight, dry weights and total leaf area and yield were recorded 25% concentrations of effluent irrigation. It may be the presence of toxic pollutants in the effluent. That kind of pollutants mainly affect the respiration of the root. Respiration of root and soil organism tends to reduce the oxygen and increase the CO$_2$ concentration. The soil becomes harder and closed the pores of the soil are closed causing less aeration and retarding the growth of plant [23].

Chlorophyll estimation is one of the important biochemical parameters which is used as the index of production capacity. The chlorophyll ‘a’, chlorophyll ‘b’, total chlorophyll and carotenoid content were analysed at 15 and 60 DAS. The 10% dilution of sugar mill effluent concentrations increased the chlorophyll and carotenoid contents (Fig. 5). It may be due to favourable effect of elements present in the effluent on pigment system [31]. Reduction in chlorophyll content induced by higher
concentrations of effluent may be associated with mineral ions [29, 30]. It may also due to the formation of enzymes chlorophyllase which is responsible for chlorophyll degradation [32].

The increase in protein content (Fig. 6) of greengram at lower concentration of sugar mill effluent was observed. It might be due to adsorption of most of the necessary elements by plants [33]. The protein content decreased with the increase of effluent concentrations. It has been reported that the presence of high concentrations of various cations and anions in the effluent. The similar result was observed in the changes induced by the effluent stress [27]. The level of amino acids decreased upto 75% concentration of effluent this may be due to the inverse relationship between protein and amino acids where protein content was increased and amino acid were low. Initial rise in amino acids content may be due to higher protease enzyme activity which suggest that proteins are in continuous state of turnover and amino acids newly incorporated into proteins are not in association. The breakdown of protein into amino acids is also adversely affected due to effluent toxicity. Hence poor availability of nitrogen may be a causative factor for reduction in protein content [34].

The amino acid content increased in 10 per cent effluent concentration and then decreased at higher concentration (Fig. 7). The decreasing activity may be due to the inhibitory effluent of the effluent on protease activity. The major amount of the enzyme appeared to be synthesized during the early stages of germination which could be stopped by an inhibitory action on protein synthesis [28].

The sugar content was found to be increased under 10 per cent concentration (Fig. 8). of sugar mill effluent and then the content decreased at higher concentrations from 25 per cent onwards. From these observation, it can be inferred that at lower effluent concentration, the reserve food material has been efficiently utilized for growth and development of seedling. The lesser sugar content was recorded in the crop at higher effluent concentrations of effluent treatments from 25 to 100 per cent concentration. It implies the deranged sugar
metabolism and poor translocation of starch and other metabolites to the growing axis [35]. The decrease at higher concentration may be due to the excessive nitrogen uptake caused imbalance between nitrogen uptake and assimilation and a large supply of nitrogen might eventually lead to depletion of carbohydrate reserve.

The antioxidant enzyme catalase play a major role in several metabolic pathway. In this study, the catalase gradually increased in shoot and root of greengram with increase in the sugar mill effluent treatment (Fig. 9). The greatest response to effluent pollutants indication of a stress and direct response to the generation of superoxidase radical by pollutants induced blockage of the electron transport chain in the mitochondria [36].

The data on the peroxidase and polyphenol oxidase activities of greengram grown in various concentrations of sugar mill effluent are presented in (Fig. 10 and 11). The figure indicate that lethal effect increased with the increase in concentration of effluent. The effluent concentration at 10 percent do not affect shoot and root peroxidase activity compared to control at the same time, the increasing effluent concentration peroxidase activity was increased, the changes of peroxidase activity could have been due to the presence of large amounts of various cations and anions in the sugar mill effluent [37,38].

**CONCLUSION**

It can be concluded that the untreated sugar mill effluent reduced the crop growth. However, the stimulation of growth and yield were observed at lower concentration of effluent. It is suggested that sugar mill effluent have to be diluted up to 10% level before it is used for irrigation. After dilution, the effluent
characteristics will become within the prescribed limits and pollution load of the effluent decreased. The effluent at lower (10%) concentration can serve as a liquid fertilizer for the cultivation of agricultural crops.

REFERENCES