ABSTRACT

Waste released from sugar factories has a huge amount of inorganic and organic matter whose effect on plant is different when used in different concentrations. Hence, the effects of sugar factory effluent on the morphological and biochemical parameters of chickpeas (Cicer arietinum Linn.) were studied by using the different concentrations of effluent for irrigation of chickpeas plant. The experiment was conducted at Botanical Garden, Department of Biochemistry, Bhaila (PG) College, Bhaila (Saharanpur), Uttar Pradesh. In the experiment, pots were arranged in a completely randomized design, replicated by six times and were labeled for the various treatments viz. 0%, 10%, 25%, 50%, 75% and 100% v/v. After that, chickpea plants were grown up to 10 days, in the soil irrigated with different concentrations of sugar factory effluent. It was observed that sugar factory effluent promoted the growth of chickpea seeds at 10–25% concentration, but its lethal impacts were noted when concentrations were above 50–100%. Hence, it was found that at lower concentrations (upto 25%), sugar factory effluent behaves as compost for better growth of chickpeas, but at high concentrations, it shows toxicity and behaves as a pollutant. Moreover, it makes the speed of biochemical reactions slow in chickpeas. Therefore, sugar factory effluent can be used for irrigation of chickpea after proper dilution.

[Keywords: Sugar factory effluent, chickpeas, morphological and biochemical parameters, toxicity]

INTRODUCTION

India is one of the largest sugar-producing countries in the world. There are 453 sugar factories throughout India. The sugar factories are playing an important role in the Indian economy. An income source to a large number of people like laborers, farmers, transport operators and technicians is provided by the sugar factories during the cultivation and transportation of sugarcane. Crystalline sugar and bioethanol are the main final product of the sugar factory which is gained from the fermentation and distillation of sugarcane juice and molasses. Sugar manufacturing factories deliver an excessive advantage to manhood, but sugar factories also create a wide range of environmental impacts.

Sugar factories release the effluent that makes aquatic and terrestrial ecosystems more polluted with organic as well as inorganic substance (Malaviya and Sharma 2011) Effluent released during sugar production in sugar factories has numerous physical agents like total dissolved solids (TDS), total suspended solids (TSS), total solids (TS), biochemical oxygen demand (BOD),
and chemical oxygen demand (COD) (Vinod and Chopra 2010). Such physical agents determine the polluting effect of an effluent. These pollution causing agents affect the photosynthetic action of rooted plants (Jiri et al. 2008). On the other hand, some chemical agents present in effluent such as heavy metals (like nickel, cobalt, cadmium, copper, lead, chromium, potassium, magnesium, and calcium) (Vinod and Chopra 2010) also determine the level of pollution in water because they inhibit photosynthesis and transpiration of plant beyond a definite concentration (Bazzaz, Carlson and Rolfe 1974). These agents cause many changes in the physico-chemical properties of water and make the water harmful for both living organisms and plants. Effluent affects not only the food crops but also deposits heavy metals in the plant tissue. Besides, it also affects the user, so the cultivation of food crops is avoided under effluent irrigation.

Although the effects of industrial effluent are hazardous, Nath, Singh and Sharma (2007) found that it was occasionally used for irrigating numerous crops (wheat, garden pea, black gram, and mustard). Additionally, it was also observed that sugar factory effluent may be beneficial at 10% for Tagetes erecta L. (Thanapal and Perumal 2017); 40% for Zea mays L. (Vinod 2014); 40% for Raphanus sativus L. (Vijayaragavan et al. 2011); 10% for okra and tomato plants (Musaa Khan, Yang and Din 2019). Moreover, they also found that sugar factory effluent is a good source of nutrition for vegetation if diluted before irrigation. Crops and soil fertility could be increased by applying environment-friendly techniques during effluent discharge. Ijaz et al. (2018) examined that the use of sugar factory effluent at a concentration of 75% in combination with plant growth-promoting rhizobacteria (PGPR) was the most effective method for improvement in stay-green, leaf emergence, growth and productivity of maize. Also, Renu, Jyoti and Anoop (2014) observed that sugar factory effluent may affect the soil properties because the effluent contains a high concentration of sulphur, organic waste, potassium, and phosphorous. Finally, they concluded that sugar factory effluent may be beneficial as well as harmful, depending upon how much concentration is being used. The effluent at high concentration disturbs the pH, N, P, K, CaCO₃ and organic matter of the soil when effluent is used for irrigation (Ajmal and Khan 1983).

A field study was conducted in the Experimental Garden of the Department of Biochemistry, Faculty of Life Sciences, Bhaila (P.G.) College, Bhaila (Saharanpur), U.P. India for studying the irrigation effect of sugar factory effluent on the level of morphological and biochemical parameters of chickpeas. Pots used for the experiment were arranged in a completely randomized design, replicated by six times and were labeled for the various treatments viz. 0%, 10%, 25%, 50%, 75% and 100% v/v.

Seed Collection and Propagation

The seeds of chickpeas were purchased from a registered seed center at Saharanpur, U.P. India. The soil used in the experiment was well-drained loam and clay loam in nature. About 3 kg of air-dried soil were taken into separate pots. Five different concentrations (viz., 0%, 10%, 25%, 50%, 75% and 100%) of sugar factory 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 500 ml of respective concentration of test solutions daily. Plants were thinned to a maximum of three per pots, after germination. Each treatment including the control was replicated five times.

**Effluent Collection**

The effluent was collected from Triveni Sugar Factory, Deoband (Saharanpur), U.P. India (10 km away from college campus) in pre-cleaned, acid washed, 5 L carboys and stored in a refrigerator below 5°C until used. The effluent collected from the sugar factory was about 4 L. After collecting it, six concentrations were made by following the dilution method ($C_1V_1 = C_2V_2$).

**Physico-Chemical Analysis of Sugar Factory Effluent**

The collected sugar factory effluent sample was examined for their several physico-chemical characteristics in Central Instrumentation Facility, Jamia Millia Islamia (A Central University) as per standard methods mentioned in APHA (2005). The collected effluent was analyzed for temperature, pH, total solids (TS), total dissolved solids (TDS), total suspended solids (TSS), chloride content, electrical conductivity, alkalinity, acidity, silica, heavy metals, oil/grease, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) values.

**Laboratory Assay of Sugar Factory Effluent**

For bioassays, the effluent was diluted to 0%, 10%, 25%, 50%, 75% and 100% v/v with distilled water. Seeds of chickpeas were sterilized with 0.1% w/v mercuric chloride solution for 5 minutes to remove microbes and then washed three times with sterile distilled water. Plant seeds were spread on each sterilized petri dish lined with blotting paper and then irrigated with 5 mL of the different concentrations of sugar factory effluent. Each treatment consisted of three replicate plates with forty seeds per plate. Observations were recorded at 24 hours intervals. The effect of different concentrations of sugar factory effluent on the 10th day was studied on the following parameters of chickpeas.

**Parameters Observed**

**Morphological Parameters**

The various morphological growth parameters such as shoot length, root length (cm/seedling), fresh weight and dry weight (g/seedling) were recorded in 10th-day plants. Germination denotes the early appearance of the radicle by the visual remark. We calculated germination using the following formula of Awasthi et al. (2016).

\[
\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total numbers of seeds sown}} \times 100
\]

**Vigor Index**

The vigor index of the seedlings was calculated through the formula given by Abdul-Baki and Anderson (1973).

\[
\text{Vigor index} = \text{Germination percentage} \times \text{Length of seedling}
\]

**Tolerance Index**

The tolerance index of the seedlings was calculated through the formula given by Turner and Marshall (1972).

\[
\text{Tolerance index} = \frac{\text{Mean length of longest root in treatment}}{\text{Mean length of longest root in control}} \times 100
\]

**Biochemical Parameters**

Chlorophyll (a, b and total chlorophyll content) and soluble protein concentration were estimated by the method of Arnon (1949) and that of Lowry et al. (1951), respectively. On the other hand, inorganic phosphate (Pi) by the method of Lowry and Lopez (1946) and protein phosphatase (Ppase) and acid phosphatase (AcPase) that of LeBel, Poirier and Beaudoin (1978) were determined.

**RESULTS AND DISCUSSION**

**Physico-Chemical Parameters of Effluent**

Physico-chemical analysis of sugar factory effluent is given in Table 1. The effluent was dusky brown in color. The reduction in some other parameters such as temperature, chemical oxygen demand, and biochemical oxygen demand is caused by coloration. Coloration may also increase the biodegradability of substances (Ingaramo et al. 2009). Organic matter on decomposition under anaerobic condition, produces various sulphides, particularly ferrous sulphide. This type of sulphide causes effluent coloration. Like other parameters, the temperature is also a very important parameter. Normally, the organisms in aquatic life rapidly grow at 20–27 °C (Ezhilvannan et al. 2011). In agriculture, the
Table 1. Physico-chemical characteristics of sugar factory effluent.

| Parameters (unit)        | Mean ± SD | Tolerance limits (TPCB 2009) |
|--------------------------|-----------|-----------------------------|
| Acidity (mg L⁻¹)         | 1571.53 ± 1.21 | -                           |
| Alkalinity (mg L⁻¹)      | 1.10 ± 2.51 | -                           |
| BOD (mg L⁻¹)             | 6772.56 ± 0.9 | -                           |
| COD (mg L⁻¹)             | 32545.52 ± 1.85 | 250                         |
| Colour                   | Dusky brown | Colorless                   |
| Electrical conductivity  (mhos cm⁻¹) | 5290.01 ± 1.55 | -                           |
| Odor                     | Rotten molasses | -                          |
| Free CO₂ (mg L⁻¹)        | 9.30 ± 0.12 | -                           |
| pH                       | 8.73 ± 0.02 | 5.5-9.0                     |
| TSS (mg L⁻¹)             | 302.45 ± 1.79 | -                           |
| Temperature (°C)         | 35.3 °C | 40 °C                        |
| TDS (mg L⁻¹)             | 2927.10 ± 2.49 | 200                         |
| TS (mg L⁻¹)              | 3172.2 ± 1.12 | 2100                        |
| Total hardness (mg L⁻¹)  | 551.09 ± 0.98 | -                           |
| Calcium (mg L⁻¹)         | 99.27 ± 1.29 | -                           |
| Chloride (mg L⁻¹)        | 851.37 ± 1.42 | 600                         |
| Copper (µg mL⁻¹)         | 153.88 ± 1.31 | 10⁻⁴                       |
| Fluoride (mg L⁻¹)        | 1.47 ± 0.33 | 1.0                         |
| Iron (µg mL⁻¹)           | 974.52 ± 0.31 | 10⁻⁴                       |
| Lead (µg mL⁻¹)           | 0.36 ± 0.1 | 5 × 10⁻⁶                    |
| Magnesium (mg L⁻¹)       | 212.18 ± 0.95 | 100                        |
| Manganese (µg mL⁻¹)      | 375.09 ± 2.07 | 0.01                       |
| Phosphorous (mg L⁻¹)     | 6.95 ± 1.41 | 10                          |
| Silica (mg L⁻¹)          | 22.32 ± 1.76 | -                           |
| Sulphate (mg L⁻¹)        | 159.7 ± 0.2 | 12                          |
| Zinc (µg m L⁻¹)          | 99.32 ± 2.31 | 10⁻⁴                       |
| Oil and grease (mg L⁻¹)  | 14.10 ± 0.12 | -                           |

Length of Germinating Seedling

The length of chickpeas seedling showed an increasing trend at sugar factory effluent concentration up to 25% (Figure 2a), and the minimum in seedling length was observed in 100% sugar factory effluent concentration. In 25% sugar factory effluent, the micro-nutrients were at optimum concentration required for better germination because above this concentration, all nutrient showed fatality, and reduced the length of germinating seedling. Hence, the results had a great significance at 25% because seedling length plays an important role in determination of seedling quality that influences early plant growth after transplanting (Ohsumi et al. 2015). Hence, the seedling quality was better when the concentration of the sugar factory effluent was 25%.

Root and Shoot Length

The root and shoot length of chickpeas showed an increasing trend at lower concentration of sugar factory effluent up to 25% (Figure 2b), while minimum seedling shoot length (8.1 cm) and root length (7.2 cm) were observed in 100% sugar factory effluent concentration. The results were very significant at 25% sugar factory effluent concentration because the length of the root is also a very important trait since it is related to nitrogen absorption (François et al. 2015). Arindam (2000) reported the reduction in the percentage of dividing cells, i.e. mitodepressive action in root tips of *Hordeum vulgare* with an increase in the concentration of sugar factory effluent.

Fresh and Dry Weights of Seedling

The maximum fresh weights of seedling root and shoot were observed 3.3 g and 3.8 g per seedling, respectively,
at 25% effluent concentration, corresponding to dry weights of 1.8 g and 2.1 g per seedling, respectively. The minimum fresh weights of root and shoot were observed 1.87 g and 1.12 g per seedling, respectively, at 100% effluent concentration, corresponding dry weights of 0.98 g and 0.92 g per seedling, respectively (Figure 2c and 2d). It has been studied that the developing seed increases considerably in volume and mass due to significant cell expansion and the accumulation and storage of proteins, lipids, and carbohydrates during maturation (Long et al. 1981; Bechtel et al. 1982; Rosenberg and Rinne 1986). Hence, the results were clearly showing their significance at 25% sugar factory effluent because the growth promoting chemical constituents such as sulphate and chlorides that are not only involved in mineral and salt formation but also stimulate the growth at lower concentration (Patterson et al. 2008) were in their finest range. Moreover, it has also been studied that the variation of fresh and dry weight is due to the presence of salt and minerals at the lower concentration and optimum level (Ayyasamy et al. 2008).

**Germination Percentage**

The germination percentage of chickpeas as affected by different concentrations of sugar factory effluent is shown in Figure 3. The minimum percentage of germination (54) and the maximum (94) was observed at 100% and 25% v/v
Effects Of Crude Sugarcane Factory effluent on ... (Mohd. Suhail et al.)

Effects Of Crude Sugarcane Factory effluent on ... (Mohd. Suhail et al.)

effluent concentrations, respectively. The results clearly showed their significance at 25% of effluent because the ecology of seeds and their germination patterns can determine range limits, adaptation to environmental variation, species diversity, and community responses to climate change (Huang et al. 2016). It may be due to the presence of micronutrient and macronutrient in the effluent (Kannan 2001). The reduction in seed germination percentage at a higher concentration of effluent may due to the higher amount of sludge present in the effluent (Nagajyoti et al. 2008; Sharma 2011). The other possibility of a reduction in germination percentage may be due to the change in tricarboxylic acid enzyme levels during germination (Firenzuoli et al. 1968) which is caused by the presence of an excess of ammonia because ammonia causes depletion of the tricarboxylic acid enzyme (Lai and Cooper 1986).

Vigor Index

The highest vigor index (1705.16) of seedlings occurred at 25% of effluent treated seedling and the lowest vigor index (663) at 100% of effluent (Figure 3). It is already understood that the seed vigor index is an important component because the larger the value of vigor index the higher will be the production of crops. Hence, keeping this fact into consideration, we can say that 25% sugar factory effluent should be recommended for being used in chickpea area. Therefore, vigor index regarding results were statistically important at 25% sugar factory effluent.

Tolerance Index

The highest tolerance index (1.98) of seedlings was observed in 25% of effluent treated seedling and the lowest tolerance index (0.39) at 100% of effluent (Figure 3). Tolerance index is an important component because it evaluates the tolerance level of plant species towards air pollution from leaf biochemical parameters such as total chlorophyll. The results clearly showed their statistically significant when the concentration of sugar factory effluent was 25%, but that sugar factory effluent had converted into pollutant for chickpeas when the concentration approached beyond 25%.

Biochemical Parameters

Chlorophyll-a, Chlorophyll-b and Total Chlorophyll Content

Maximum photosynthetic pigment such as chlorophyll a, chlorophyll b and total chlorophyll of chickpeas were observed at 25% concentration of sugar factory effluent in the soil. Chlorophyll concentration in leaves is an indicator of plant health (Porra 2002). Hence, we can say that the results were significant when the concentration used was 25%. Further, the chlorophyll a:b ratio also indicates the developmental state of the photosynthetic apparatus in plants. It has a determinative role in the growth and development of higher plants. The chlorophyll content also indicates the photosynthetic capacity per unit area of the leaf (Kozlowski et al. 1991) that determines the rate of photosynthesis in the plant (Dickmann and Kozlowski 1968).

The increased chlorophyll content was obviously due to sugar factory effluent at low concentrations which act as structural and catalytic components of protein enzymes and as cofactors for normal development of chlorophyll biosynthesis. In higher concentrations of sugar factory effluent (50%, 75% and 100% v/v), they become toxic to plants and a decrease in photosynthetic pigments was observed (Figure 4a). Our results align with the finding of Nagajyoti et al. (2008) who reported that the increased chlorophyll content at lower concentration maybe 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 as proposed by Swaminathan and Vaidheeswaran (1991).

Total Protein, Protein Phosphatases and Acid Phosphatases

The increase in total protein, protein phosphatases and acid phosphatases in the whole seedling (Figure 4b), root (Figure 4c) and shoot (Figure 4d) of chickpeas were observed at 25% concentration of sugar factory effluent. The results at 25% were very important with a great significance because plants store proteins in the embryo and vegetative cells to provide carbon, nitrogen, and sulfur resources for subsequent growth and development (Herman and Larkins 1999). Moreover, the deposited protein offers building blocks for quick progress upon seed and pollen growth. Likewise, protein reserves in vegetative cells deliver the building blocks for seed and fruit set during reproductive growth and for rapid expansion of vegetative structures after periods of dormancy. Hence, 25% of effluent sugar factory effluent was sufficient for providing micro-nutrient required for protein synthesis in chickpea.

On the other hand, the results were also very important in case of phosphatase activity determination at 25% because the secreted APases of roots and cell cultures are relatively non-specific enzymes that appear to be...
important in the hydrolysis and mobilization of Pi from extracellular phosphomonoesters for plant nutrition (Duff, Sarath and Plaxton 1994). The total protein, protein phosphatases, and acid phosphatases activity showed a decreasing trend at higher concentrations of 50–100% of the sugar factory effluent. The effect may be due to the transportation of most of the nitrogen absorbed by the plants (Glass et al. 1999). Lenin and Thamizhiniyan (2009) reported the significant increase in the protein content of chickpeas might be due to the potassium and nitrogen in their optimum quantity present in the lower concentration of the effluent. This may be due to the optimum levels of the micro and macronutrients present in the effluent that may serve as an additional potential source of liquid fertilizer favorable to the seedling development (Asgharpour and Reza Azizmoghaddam 2012).

CONCLUSION

The effluent with the highest concentration inhibited both seedling growth and seed germination of chickpeas. The maximum percentage of germinating seedling growth, fresh and dry matter production, total chlorophyll and protein contents, protein phosphatases and acid phosphatases occurred at 10–25% v/v sugar factory effluent. Concentration at 10–25% v/v was suitable for the seed germination and biochemical changes and mineral contents of chickpeas seedling. Hence, the harmful effects of sugar factory effluents may be converted into a useful one by making it dilute up to 25% v/v, especially for chickpeas. Therefore, sugar factory effluent can be used for irrigation after proper dilution for chickpeas cultivation.

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DISCLOSURE STATEMENT

The authors have no conflict of interest.

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