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Distillery Effluent Induced Changes in the Growth and Pigment Content in *Trigonella foenum-graecum* L.

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ABSTRACT

A byproduct of sugar industry, the molasses is used as a raw material for the production of alcohol and it also generates a large amount of waste water, the effluent. In the present investigation, an attempt has been made to assess the effect of distillery effluent on the growth and pigment content of *Trigonella foenum-graecum* L. The physico-chemical characters were studied and it was observed that distillery effluent is highly acidic, reddish brown color, high values of EC, TDS, sodium, chloride, sulfate, bicarbonate, BOD and COD and is devoid of dissolved oxygen. Germination efficiency of seeds of *Trigonella foenum-graecum* L. was measured by treating seeds with different concentrations (20%, 40%, 60% and 80% (v/v)) of effluent along with control using ground water. The same concentrations were applied to 10 days old seedlings grown in pots. On the 21st day after sowing, the seedlings were collected and observed that all growth parameters like germination percentage, root length, shoot length, fresh weight, dry weight and vigor index was maximum in 20% concentration of the distillery effluent and it was significantly reduced in higher effluent concentrations (40%,60% and 80%) when compared to control. Beyond that no germination was observed in 100% effluent concentration. The chlorophyll content was found to be decreased as the concentration of the effluent increased except in 20% (v/v) concentration. Phytotoxicity was absent in 20% (v/v) effluent concentration but maximum in 80% (v/v) effluent concentration. Finally it was observed that the effluent can be used for irrigation purpose after proper dilution with ground water.

Keywords Distillery effluent, *Trigonella foenum-graecum* L., Growth parameters, Pigment content.

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INTRODUCTION

Development of a nation is usually seen through its industrial development. Modern technologies elevated the nation globally and also for its economic development which further results in a very big threat to the environment. India satisfies the global needs of sugar by its huge production through nearly 579 sugar mills. Molasses a byproduct of sugar industry is being used as a raw material in distilleries and at present nearly 285 distillery units are functioning in India. Annually, these distillery industries produce 2.71 billion liters of alcohol and generating 40 billion liters of waste water. Distilleries releases enormous amount of waste water known as spent wash which is around 80% of the raw material to the environment

(Farid et al. 2012). Distillery effluents contain high volume of organic and inorganic nutrients and have been reported to have a beneficial effect on some crop yields as well as inhibitory effect on other crop yields (Jadhav et al. 2010). In a country like India, to obvercome water scarcity, the farmers are forced to use the industrial effluents as a source of irrigation for the crops. Like that, distillery efflunt is also being used by the farmers unaware of the toxicity and without keeping in mind the fact that the untreated raw effluent use may lead to disastrous effect on the growth and quality of the crops. Hence the present study reports on the utilization of distillery effluent on growth and pigments content of *Trigonella foenum -graecum* L.

MATERIALS AND METHODS

Effuent collection

The distillery effluent was collected directly from the outlet of the industry which was used by the farmers of that area for irrigation purposes.

Physico-chemical analysis of the distillery effluent

The effluent was stored and all the analytical processes were carried out according to the standard method of APHA (2012). The physico-chemical characters such as pH, BOD, COD, suspended, dissolved solids and total dissolved solids potassium, phosphorus and sulfate were analyzed.

Seed source

Healthy and viable seeds of *Trigonella foenum-graecum* L. were procured from seed vendor, certified by Tamilnadu Seed Certification Department, Virudhunagar, Virudhunagar District.

Seedling treatment

Healthy seeds of *Trigonella foenum-graecum* L. were surface sterilized with 0.1% mercuric chloride for one minute and washed with running tap water followed by distilled water. Various concentration of the distillery effluent was prepared such as 20%, 40%, 60% and 80% (v/v). Seeds were soaked in distilled water for 2 h . The seeds were allowed to grow in pots containing 9 kg of uniformly mixed red, black and sandy soil 1 : 1 : 1 ratio. The experimental sets were kept in diffused sunlight at room temperature. The experimental troughs were watered everyday with the respective concentrations of the effluent (750 ml). The control sets were maintained with tap water. Both experimental sets and control sets were maintained in triplicates. On the 21st day, the seedlings were plucked out without any damage and analyzed for the growth parameters and pigment content. The germination percentage, root length, shoot length, fresh weight and dry weight were estimated as per Arts and Marks (1971). The vigor index was calculated by multiplying germination percentage with total length of seedlings (Abdul and Anderson 1973). Further the pigments content such as Chlorophyll-*a*, Chlorophyll-*b,* total chlorophyll and carotenoids were quantitatively estimated (Wellburn and Lichtenthaler 1984).

RESULTS AND DISCUSSION

The physico-chemical characteristics of distillery effluent were presented in Table 1. In the present investigation, it was observed that the distillery ef-

Table 1. The physico-chemical characteristics of the distillery effluent. All the values are expressed in mg/l except temperature, pH and electrical conductivity (micromhos/cm).

Sl. No.	Parameters	Values /Characters
1	pH	4.2
2	Color	Dark brown
3	Temperature	30^{o}C
4	Electrical conductivity	16450
5	Total solids	42400
6	Total dissolved sloilds	36200
7	Total suspended soilds	6200
8	Total hardness	2432
9	Salinity	2864
10	Sodium	580
11	Potassium	6210
12	Calcium	2070
13	Magnesium	2260
14	Sulfate	5000
15	Chloride	8530
16	Nitrogen	1254
17	Phosphate	44
18	Dissolved oxygen	Nil
19	COD	32300
20	BOD	57164

Table 2. Effect of distillery effluent on the Germination percentage and Germination index of *Trigonella foenum-graecum* L.

Sl. No.	Parameters	Control (water)	20% (v/v)	40% (v/v)	60 (v/v)	80 (v/v)
1	Germination percentage (%)	90	94	70	50	30
2	Germination index (%)	100	122	95	36	17

fluent was highly acidic (ph 4.2), reddish brown color and contained high value of electrical conductivity (16450 mmhos/cm), suspended solids (6200 mg/l), dissolved solids (36200 mg/l) and total solids (42400 mg/l) which is an indication of pollution. A very high value of total hardness, calcium and magnesium was also noted. The range of dissolved oxygen was nil and it may be due to high organic load. A very high value of BOD (32300 mg/l), COD (57164 mg/l) was also noted. Distillery effluent is of purely plant origin and contains large quantities of plant nutrients suchas sodium, potassium, phosphorus and sulfate which the sugarcane plant has absorbed from the soil. In contrary the distillery effluent is devoid of dissolved oxygen and also heavy metals.

Effects of various concentrations of distillery effluent on the germination percentage and vigor index of *Trigonella foenum-graecum* L. were presented in Table 2. The petridish study of germination percentage showed higher percentage in 20%(v/v) concentration and the percentage was gradually decreasing from 40% up to 80% (v/v) concentrations and no germination was observed in 100% effluent concentration. This decrease may be due to the disturbance of the osmotic relations of the seed and effluent water, thus reducing the amount of water absorption and retarding seed germination. Agarwal et al. (2010) also reported a reduction in germination at higher conccentration of the distllery effluent treated seedling of wheat, mung bean and pea. The increase in germination percentage at lower concentration of the effluent might be due to the fertilizing effect at lower concentration of the effluent and optimum conditions provided for seed germination. Our results are homogenous to the findings of Pandey et al. (2008) on maize and rice in distillery effluent and Vinod and Chopra (2014) in *Trigonella foenum -graecum* irrigated with paper mill effluent. In the present study, the vigor index of the effluent treated seedlings showed a gradual decrease with increase in concentration (Table 2) which is similar to the earlier findings of Parvathi et al. (2014) in *Cicer arietinum.*

Other growth characteristics were measured from the seedlings grown 21 days after sowing . Effects of various concentrations of distillery effluent on the growth of *Trigonella foenum-graecum* L. were presented in Table 3. A slight increase in shoot and root length was observed in 20% concentration and starts declining with increasing concentration of the distillery effluent. It may be attributed to the presence of nutrients like P,Ca, K and Mb. At higher concentrations of treated distillery effluent both root and shoot lengths were affected. An elevated amount of nutrients at higher concentration of the effluent probably become toxic resulted in the inhibition of growth parameters. A similar hike in root and shoot length in *Brassica* at 25% concentration of the distillery effluent and a decrease in root and shoot length

Table 3. Effect of distillery effluent on the growth of *Trigonella foenum-graecum* L. Values in parentheses indicate percent activity with respect to control. Value represents mean of 5 samples with their standard error (±).

Sl. No.	Characters	Concentration of distillery effluent				
		Control (water)	20% (v/v)	40% (v/v)	60 % (v/v)	80 % (v/v)
1	Shoot length (cm)	12.5 ± 0.145 (100)	13.9 ± 0.271 (111)	11.6 ± 0.285 (93)	10.8 ± 0.351 (86)	8.9 ± 0.208 (71)
2	Root length (cm)	5.6 ± 0.189 (100)	6.5 ± 0.174 (116)	5.3 ± 0211 (95)	3.6 ± 0.135 (64)	2.8 ± 0.014 (50)
3	Fresh weight (g)	1.7 ± 0.096 (100)	1.82 ± 0.161 (107)	1.36 ± 0.092 (80)	1.13 ± 0.049 (66)	0.96 ± 0.031 (56)
4	Dry weight (g)	1.17 ± 0.031 (100)	1.26 ± 0.029 (108)	1.01 ± 0.029 (86)	0.92 ± 0.034 (79)	0.58 ± 0.045 (50)

Table 4. Effect of distillery effluent on the pigment content of *Trigonella foenum-graecum* L. Values in parentheses indicate percent activity with respect to control. Value represents mean of 5 samples with their standard error (±).

Sl. No.	Parameters	Concentration of distillery effluent				
		Control (water)	20% (v/v)	40% (v/v)	60 % (v/v)	80 % (v/v)
1	Chl-*a* (mg/g LFW)	0.39 0.017 (100)	0.42 ± 0.017 (107)	0.34 ± 0.025 (87)	0.29 ± 0.021 (74)	0.21 ± 0.021 (53)
2	Chl-*b* (mg/g LFW)	0.14 ± 0.031 (100)	0.17 ± 0.01 (121)	0.11 ± 0.015 (79)	0.09 ± 0.015 (64)	0.06 ± 0.006 (43)
3	Total Chlorophyll (mg/g LFW)	0.53 ± 0.042 (100)	0.59 ± 0.021 (111)	0.45 ± 0.042 (85)	0.38 ± 0.025 (72)	0.27 ± 0.021 (51)
4	Carotenoids (mg/g LFW)	0.56 ± 0.026 (100)	0.63 ± 0.026 (112)	0.52 ± 0.025 (93)	0.45 ± 0.04 (80)	0.36 ± 0.026 (64)

at higher concentrations was reported by Richa et al. (2016). Total biomass is the best indicator of response to the distillery effluent. It reflects the long-term integration on the biochemical processes that affects plant growth. The present study revealed that in *Trigonella foenum-graecum* L. a slight increase in fresh weight and dry weight was observed in 20% concentration and starts declining with increasing concentration of the distillery effluent which coincides with the findings of Basavaraju and Chandraju (2008) , Narain et al. (2012) in *Cicer arietinum.*

The presence of optimum level of nutrients in the lower concentration of the effluent might have increased both fresh weight and dry weight and a reduction in dry weight of seedlings in higher concentration indicated a disturbed metabolism of the seedlings. The germinating seedlings under higher concentrations would get fewer amounts of oxygen due to organic and inorganic chemicals present in the effluent and further a reduction in biomass was observed in brinjal (Rajesh et al. 2016) irrigated with textile effluent and in *Cicer arietinum* (Chaudhary et al 2016) irrigated with paper mill effluent. A similar trend was reported by Saratha et al. (2018) in *Capsicum annuum* irrigated with plate making effluent and (Mathan et al. 2019) in *Acacia holosericea* irrigated with winery waste water.

Pigment content analysis

Effect of various concentrations of distillery effluent on the pigment content of *Trigonella foenum-graecum* L. is revealed in the Table 4. In the present study, a slight hike in the value of Chl-*a*, Chl-*b* and total chlorophyll was observed in 20% (v/v) concentration of the effluent and it might be due to the presence of significant content of Fe, Mg and Mn in the distillery effluent required for the synthesis of chlorophyll in plants which is correlated with the findings of Singh and Swami (2014) in *Vigna radiata.*

Reduction in the pigments content was observed when the concentration of the effluent was increased from 40% up to 80% (v/v). Reduction in chlorophyll induced by higher concentration of effluent may be associated with mineral ions. It may also due to the formation of enzymes chlorophyllase which is responsible for chlorophyll degradation (Khan et al 2011). The inhibition of chlorophyll synthesis may be due to the induced inhibition of electron transport in Photosystem (PS) II. Increasing concentration of waste water is inhibitory to synthesis of chlorophyll molecules (Baskaran et al. 2009).

In the present investigation, although a slight hike in the carotenoid content was observed in 20% (v/v) effluent concentration, a sharp decline was noted with other higher concentration of the effluent. These results are in agreement with those of Vinod (2016) in *Trigonella foenum-graecum* irrigated with sugar mill effluent. A decrease in carotenoid content at higher concentration of textile effluent was also reported by Garg aand Kaushik (2008) in sorghum and on turnip, raddish and on *Capsicum annuum* irrigated with plate making effluent (Saratha et al. 2009).

Effluent discharged from distillery industries is rich in plant nutrients and dilution of distillery efflu-

ent up to 20% reduces the nutrients present in it and results in enhancement of growth parameters in the present study. However at higher concentrations of the effluent, the elevated level of nutrients probably becomes toxic resulting in inhibition of morphometric characters and diminution of pigment content. In the current scenario, it was advisable that the farmers could utilize the distillery water only after appropriate dilution for irrigation and to promote sustainable agriculture.

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