Pearl millet (*Pennisetum Glaucum* L.) response after ferti-irrigation with sugar mill effluent in two seasons

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**Abstract**

**Background** The disposal of sugar mill effluent has become a major problem in India due to generation of huge volume of effluent. The value of wastewater for crop production has been recognized in many countries, including India. The effluents not only contain nutrients that stimulate growth of many crops, but also may have various toxic chemicals, metals, metallic oxides along with nitrogenous and phosphate compounds, which may affect various agronomical characteristics of crop plants. The present investigation was conducted to assess the agro-potentiality of agro-based sugar mill effluent as ferti-irrigant, and an alternative of irrigation water. Six plots were selected for six treatments of sugar mill effluent viz. 0 % (control), 20, 40, 60, 80, and 100 % for the fertigation of *Pennisetum glaucum* L., cv. Nandi 35. *P. glaucum* was grown, fertigated with effluent till harvest and effect of effluent fertigation on the soil and agronomical characteristics of *P. glaucum* were analyzed.

**Results** The fertigant concentration produced changes in electrical conductivity (EC), pH, organic carbon (OC), sodium (Na⁺), potassium (K⁺), calcium (Ca²⁺), magnesium (Mg²⁺), total Kjeldahl nitrogen (TKN), phosphate (PO₄³⁻), sulfate (SO₄²⁻), iron (Fe), cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), and zinc (Zn) of the soil in both seasons. The agronomic performance of *P. glaucum* increased from 20 to 40 % in both seasons compared to controls. The accumulation of heavy metals increased in soil and *P. glaucum* from 20 to 100 % sugar mill effluent concentrations in both seasons. Biochemical components like crude proteins, crude fiber, and crude carbohydrates were found maximum with 40 % sugar mill effluent in both seasons. The contamination factor (Cf) of various metals were in the order of Mn > Zn > Cu > Cd > Cr for soil and Mn > Zn > Cu > Cr > Cd for *P. glaucum* in both seasons after fertigation with sugar mill effluent. Sugar mill effluent irrigation increased nutrients in the soil and affected the growth of *P. glaucum* in both seasons.

**Conclusions** It appears that sugar mill effluent can be used as a biofertigant after appropriate dilution to improve yield of *P. glaucum*.

**Keywords** *Pennisetum glaucum* · Sugar mill effluent · Fertigation · Heavy metals · Rainy and summer season

**Nomenclature**

| Symbol | Unit          |
|--------|--------------|
| °C     | Degree centigrade |
| cm     | Centimeter    |
| dS cm⁻¹ | Desi Siemens per centimeter |
| g      | Gram         |
| gm cm⁻³ | Gram per cubic centimeter |
| kg ha⁻¹  | Kilogram per hectare |
| ml     | Milliliter   |
| mg L⁻¹  | Milligram per liter |
| mg Kg⁻¹ | Milligram per kilogram |
| mg (g f wt)⁻¹ | Milligram per gram fresh weight |
| m²     | Square meter |
| %      | Percentage    |
| NTU    | Nephelometric turbidity unit |

**Introduction**

India is one of the largest producers of sugar in the world and presently has nearly 650 sugar mills that produce about...
15 million tons of sugar and 13 million tons of molasses (spent wash) per year (Roy et al. 2007; Ezhilvannan et al. 2011). The sugar mill effluent is mainly discharged from floor, wastewater, and condensate water formed by leakage (Rathore et al. 2000; Ezhilvannan et al. 2011). The disposal of polluted wastewater is one of the main problems of today to be faced in the future with its increased adverse effects (Bharagava et al. 2008; Moazzam et al. 2012). Most of the sugar mills are discharging their effluent into the environment without any treatment (Borole and Patil 2004). It has also been reported that sugar mill effluent contains a high magnitude of pollution load and caused adverse effects on soil and biological system (Arindam and Prasad 1999; Ayyasamy et al. 2008). The effluent constitutes a number of physico–chemical elements of suspended and dissolved solids with the high amount of biological oxygen demand (BOD), chemical oxygen demand (COD), chlorides, sulfate, nitrates, calcium, magnesium, and metals (Rathore et al. 2000; Roy et al. 2007).

In addition to that, some traceable amount of heavy metals such as zinc, copper, and lead is usually present in the sugar mill effluent. The presence of these chemicals in large quantities in the effluent not only affects plant growth but also collapses the soil properties when used for irrigation (Al-Jaloud et al. 1995; Roy et al. 2007; Ayyasamy et al. 2008). Therefore, the effluent can be applied for productive uses since it contains nutrients that have the potential for use in agriculture (Kumar and Chopra 2012; Chopra et al. 2012). In agriculture, irrigation water can affect soil characteristics and agricultural crop growth (Almodares and Sharif 2007; Kumar and Chopra 2010). Besides, the use of effluent reduces fertilizer and irrigation water cost as it is available without any cost and is rich in various plant nutrients (Kumar and Chopra 2012).

Irrigation with effluents increases accumulation of metals in soil, and increases chances of their entrance in food chain (Chopra et al. 2009). Thus, contamination of agricultural soils with metals can pose long-term environmental problems, and is not without health implications (Ferguson 1990; Chopra et al. 2009). The amounts of metals mobilized in the soil environment is a function of pH, clay content, organic matter, cation exchange capacity, and other soil properties making each soil unique in terms of pollution management (Baruah et al. 1993; Kumar and Chopra 2012). The metals Cu, Fe, Ni, Zn, and other trace elements are important for proper functioning of biological systems, and their deficiency, or excess, could lead to a number of disorders (Chopra et al. 2009). Metals are capable of forming insoluble complex compounds with soil organic matter, and contents of Cd, Cu, Ni, Mn, and Zn are dependent on the pH of soil solution and soil organic matter (Kim and Kim 1999). Soil type is one of the most important factors to determine the metals’ content of food plants (Itanna 2002; Roy et al. 2007). Metals’ content in plants can also be affected by application of fertilizers or irrigation with effluent (Ferguson 1990; Ayyasamy et al. 2008).

Pearl millet is grown on more than 29 million ha in the arid and semi-arid tropical regions of Asia, Africa, and Latin America. Pearl millet has about the same nutritive quality as corn for domestic animals (Oyen and Andrews 1996; Moazzam et al. 2012). Thus, it is widely used for food and fodder all over the world and is considered as fifth most important cereal crop after wheat, maize, rice, and barley. Pearl millet biomass is variously used for the production of energy, fiber, or paper, as well as for syrup and animal feed in several regions (Radhouane 2008; Yadav and Bidinger 2008). In India, pearl millet is the fourth most important food crop, and is mostly cultivated in the northwestern states of the country. India is the largest producer of pearl millet in Asia, both in terms of area (about 9 million ha) and production (8.3 million tons) with an average productivity of 930 kg/ha during the past three years. From the early 1980s, the pearl millet area in India declined by 22%, due to inappropriate irrigation facilities (Moazzam et al. 2012). It has the ability to tolerate and survive under adverse conditions of intermittent and continuing drought (Yadav and Bidinger 2008). Therefore, pearl millet has received considerable attention during the last years as an alternative source for food, fodder, and energy production (Oyen and Andrews 1996).

In some reports, characteristics of the effluent of industries and agronomic properties of various crop plants have been determined (Hill et al. 1999; Nadia El-Sawaf 2005; Mendoza et al. 2006; Sakellariou et al. 2007; Moazzam et al. 2012). Most studies were conducted on few agronomic stages with limited parameters in various crops, but there are few reports on comprehensive agronomic studies at various agronomic stages of these plants (Kaushik et al. 2004). Use of industrial effluents on cultivation of *P. glaucum* is receiving attention (Moazzam et al. 2012) but additional information is required on how this crop responds to various concentrations of different types of effluents. The investigation was undertaken to study responses of *P. glaucum* ferti-irrigated with sugar mill effluent.

**Materials and methods**

**Experimental design**

A field study was conducted at the Experimental Garden of the Department of Zoology and Environmental Sciences, Faculty of Life Sciences, Gurukula Kangri University Haridwar, India (29°55’10.81” N and 78°07’08.12” E), to determine the effects of ferti-irrigation with sugar mill
effluent on *P. glaucum*. Six plots (each plot had an area of 9 m²) were selected for six treatments of sugar mill effluent viz. 0 % (control), 20, 40, 60, 80, and 100 % for the cultivation of *P. glaucum*. The six treatments were placed within each of the six blocks in a randomized complete block design.

Sowing of seeds of *P. glaucum*

Seeds of *P. glaucum* were sown at the end of April 2010 and 2011 for the summer season crop and at the end of July 2010 and 2011 for the rainy season crop. Seeds of *P. glaucum*, cv. Nandi 35, were procured from Indian Council of Agriculture Research (ICAR), Pusa, New Delhi, and sterilized with 0.01 % mercuric chloride, and soaked in water for 12 h. Seeds were sown in 10 rows with a distance of 30.0 cm between rows, while the distance between the seeds was 15 cm. The thinning was done manually after 15 days of germination to maintain the desired plant spacing and to avoid competition between plants.

Effluent collection and analysis

The effluent samples were collected from the R.B.N.S. Sugar mill, Laksar, Haridwar (Uttarakhand), which produces sugar from sugar cane at the rate of 150 ton sugar per day. Effluent collected from a settling tank was installed in the campus by the sugar mill to reduce BOD and solids from the effluent in plastic containers. It was brought to the laboratory and digested in an electrically heated block for 1 h at 145 °C. To this mix, 4 ml of HClO₄ was added and heated to 240 °C for 1 h. The mixture was cooled and filtered through Whatman # 42 filter paper and made with 50 ml and used for analysis. Heavy metals were analyzed using an Atomic absorption spectrophotometer (PerkinElmer, Analyst 800 AAS, Gen Tech Scientific Inc., Arcade, NY) following methods of APHA (2005) and Chaturvedi and Sankar (2006). The contamination factor (Cf) for heavy metals accumulated in sugar mill effluent irrigated soil and *P. glaucum* was calculated following Håkanson (1980).

Irrigation pattern, soil sampling, and analysis

The soil in each plot was fertigated twice in a month with 50 gallons of sugar mill effluent with 20, 40, 60, 80, and 100 % along with bore well water as the control. The soil was analyzed prior to planting and after harvest for various physico-chemical parameters: bulk density (BD), water holding capacity (WHC), soil texture, soil pH, EC, OC, Na⁺, K⁺, Ca²⁺, Mg²⁺, TKN, nitrate (NO₃⁻), PO₄³⁻, SO₄²⁻, Fe, Cd, Cr, Cu, Mn, Zn, standard plate count (SPC), and most probable number (MPN) following standard methods (APHA 2005; Chaturvedi and Sankar 2006) and used as fertigant.

Study of crop parameters

The agronomic parameters at different stages (0–90 days) were determined following standard methods for seed germination, plant height, root length, number of leaves per plant, number of tillers, spikes length, and crop yield (Radhouane et al. 2008); fresh and dry weight (Milner and Hughes 1968); chlorophyll content (Porra 2002); relative toxicity (RT) (Chapagain 1991); leaf area index (LAI) (Denison and Russotti 1997); and harvest index (HI) (Sinclair 1998). The nutrient quality of the crop was determined by using the following parameters: crude protein (4.204 Anonymous 1980), crude fiber (4.601 Anonymous 1980), and the total carbohydrate in dry matter was determined by the anthrone reagent method (Cerning and Guilhot 1973).

Extraction of metals and their analysis

For heavy metal analysis, a 5–10 ml sample of sugar mill effluent, and 0.5–1.0 g of air dried soil or plants were digested in tubes with 3 ml of conc. HNO₃ digested in an electrically heated block for 1 h at 145 °C. To this mix, 4 ml of HClO₄ was added and heated to 240 °C for 1 h. The mixture was cooled and filtered through Whatman # 42 filter paper and made with 50 ml and used for analysis. Heavy metals were analyzed using an Atomic absorption spectrophotometer (PerkinElmer, Analyst 800 AAS, Gen Tech Scientific Inc., Arcade, NY) following methods of APHA (2005) and Chaturvedi and Sankar (2006). The contamination factor (Cf) for heavy metals accumulated in sugar mill effluent irrigated soil and *P. glaucum* was calculated following Håkanson (1980).

Data analysis

Data were analyzed with SPSS (ver. 12.0, SPSS Inc., Chicago, Ill.). Data were subjected to two-way ANOVA. Duncan’s multiple range test was also performed to determine that the difference was significant or non significant. Mean standard deviation and coefficient of correlation (*r* value) of soil and crop parameters with effluent concentrations were calculated with MS Excel (ver. 2003, Microsoft Redmond Campus, Redmond, WA) and graphs produced with Sigma plot (ver. 12.3, Systat Software, Inc., Chicago, IL).

Results and discussion

Characteristics of effluent

The values of physico–chemical and microbiological parameters varied over sugar mill effluent concentrations (Table 1). The sugar mill effluent was highly alkaline with pH 8.98. The alkaline nature of the sugar mill effluent might be due to the presence of higher concentrations of...
alkalies used in the sugar manufacturing process. The values of BOD, COD, Cl\(^{-}\), Ca\(^{2+}\), Fe\(^{2+}\), TKN, SO\(_4^{2-}\), MPN, and SPC were recorded above the prescribed limits of the Indian Irrigation Standards (BIS 1991). Higher values of BOD and COD might be due to the presence of high oxidizable organic matter and rapid consumption of dissolved inorganic materials. The higher bacterial load (SPC and MPN) in sugar mill effluent might be due to the presence of more dissolved solids and organic matter in sugar mill effluent as earlier reported by Kumar and Chopra (2010). The values of TKN, PO\(_4^{3-}\), K\(^{+}\), Ca\(^{2+}\), and Mg\(^{2+}\) in the effluent were higher than the prescribed standards. In the

| Parameter | Sugar mill effluent concentration (%) | BIS for irrigation water |
|-----------|----------------------------------------|-------------------------|
| TDS (mg L\(^{-1}\)) | 198.50, 1450.00, 2942.00, 4364.00, 5668.00, 6182.00 | 1,900 |
| Turbidity (NTU) | 4.46, 1.163, 16.58, 23.47, 29.87, 33.42 | 10 |
| EC (dS cm\(^{-1}\)) | 0.34, 2.28, 4.56, 6.79, 8.88, 9.79 | 5.5–9.0 |
| pH | 7.50, 7.78, 7.87, 7.98, 8.82, 9.85 | NIL |
| DO (mg L\(^{-1}\)) | 8.24, 4.33, 3.62, 2.44, 2.11, 0 | NIL |
| BOD (mg L\(^{-1}\)) | 3.83, 180.36, 366.89, 775.68, 1230.96, 1632.58 | 100 |
| COD (mg L\(^{-1}\)) | 5.88, 234.68, 572.45, 1136.78, 1698.78, 2268.96 | 250 |
| Cl\(^{-}\) (mg L\(^{-1}\)) | 15.68, 140.52, 335.84, 652.47, 944.58, 1248.82 | 500 |
| HCO\(_3^{-}\) (mg L\(^{-1}\)) | 282.00, 310.15, 332.14, 375.48, 510.23, 662.14 | 500 |
| CO\(_3^{2-}\) (mg L\(^{-1}\)) | 105.75, 132.47, 170.85, 189.69, 214.36, 248.59 | 500 |
| Na\(^{+}\) (mg L\(^{-1}\)) | 9.65, 34.36, 80.25, 138.71, 210.58, 280.96 | 500 |
| K\(^{+}\) (mg L\(^{-1}\)) | 5.54, 40.58, 98.67, 182.47, 268.37, 349.82 | 500 |
| Ca\(^{2+}\) (mg L\(^{-1}\)) | 23.46, 110.26, 248.93, 444.45, 652.39, 842.35 | 500 |
| Mg\(^{2+}\) (mg L\(^{-1}\)) | 15.68, 140.52, 335.84, 652.47, 944.58, 1248.82 | 500 |
| TKN (mg L\(^{-1}\)) | 24.27, 42.54, 63.88, 94.37, 110.24, 141.24 | 500 |
| NO\(_3^{-}\) (mg L\(^{-1}\)) | 25.17, 104.74, 236.55, 422.87, 495.64, 788.69 | 500 |
| PO\(_4^{3-}\) (mg L\(^{-1}\)) | 0.04, 34.36, 80.25, 138.71, 210.58, 280.96 | 500 |
| SO\(_4^{2-}\) (mg L\(^{-1}\)) | 17.64, 114.12, 252.36, 464.85, 684.36, 892.87 | 500 |
| Fe\(^{2+}\) (mg L\(^{-1}\)) | 0.28, 7.36, 14.86, 21.48, 28.44, 32.69 | 500 |
| Zn (mg L\(^{-1}\)) | 0.06, 4.79, 9.63, 13.69, 20.26, 23.24 | 500 |
| Cd (mg L\(^{-1}\)) | 0.01, 1.86, 3.12, 5.24, 6.48, 8.36 | 500 |
| Cu (mg L\(^{-1}\)) | 0.04, 1.55, 2.98, 3.99, 5.78, 6.96 | 500 |
| Mn (mg L\(^{-1}\)) | 0.02, 3.45, 6.98, 8.25, 10.22, 12.48 | 500 |
| Cr (mg L\(^{-1}\)) | 0.01, 0.18, 0.39, 1.21, 1.42, 1.66 | 500 |
| SPC (SPC ml\(^{-1}\)) | 4.8 × 10\(^{3}\), 5.77 × 10\(^{3}\), 4.96 × 10\(^{3}\), 7.66 × 10\(^{3}\), 8.58 × 10\(^{3}\), 6.69 × 10\(^{3}\) | 5,000 |
| MPN (MPN 100 ml\(^{-1}\)) | 2.4 × 10\(^{2}\), 4.46 × 10\(^{4}\), 5.75 × 10\(^{4}\), 6.85 × 10\(^{4}\), 7.77 × 10\(^{4}\), 6.69 × 10\(^{4}\) | 5,000 |

Table 2 ANOVA for effect of sugar mill effluent on soil characteristics

| Source         | WHC | BD  | EC  | pH  | OC  |
|----------------|-----|-----|-----|-----|-----|
| Season (S)     | ns  | ns  | ns  | ns  | *   |
| SME concentration (C) | ns  | ns  | **  | *   | ***|
| Interaction S × C | ns  | ns  | *   | *   | ** |

SME sugar mill effluent

ns, *, ** Non-significant or significant at \( P \leq 0.05 \) or \( P \leq 0.01 \), ANOVA

Table 3 ANOVA for effect of sugar mill effluent on concentrations of cations in soil

| Source         | Na\(^{+}\) | K\(^{+}\) | Ca\(^{2+}\) | Mg\(^{2+}\) | Fe\(^{2+}\) |
|----------------|-----------|-----------|------------|------------|------------|
| Season (S)     | *         | *         | *          | *          | *          |
| SME concentration (C) | *         | *         | *          | *          | *          |
| Interaction S × C | **        | **        | **         | **         | **         |

SME sugar mill effluent

*, ** Significant at \( P \leq 0.05 \) or \( P \leq 0.01 \)
Effects of sugar mill effluent concentration and season interaction on physico-chemical characteristics of soil used in the cultivation of *P. glaucum* in both seasons

| Season × %  | EC (dS m\(^{-1}\)) | pH | OC (mg kg\(^{-1}\)) | Na\(^{+}\) (mg kg\(^{-1}\)) | K\(^{+}\) (mg kg\(^{-1}\)) | Ca\(^{2+}\) (mg kg\(^{-1}\)) | Mg\(^{2+}\) (mg kg\(^{-1}\)) |
|-------------|-------------------|---|-------------------|-------------------|-------------------|-------------------|-------------------|
| Rainy       |                   |    |                   |                   |                   |                   |                   |
| 0           | 1.92              | 7.63 | 0.52            | 25.48            | 164.33            | 16.52             | 23.43             |
| 20          | 2.98              | 7.82 | 3.68*            | 33.96*            | 188.55*           | 29.67*            | 36.86*            |
| 40          | 5.66*             | 8.01 | 6.34**           | 60.85*           | 237.94**           | 64.88**           | 57.96**           |
| 60          | 7.78*             | 8.26* | 10.92**         | 77.86*           | 265.68**           | 98.65**           | 70.36**           |
| 80          | 8.86*             | 8.45* | 15.88**         | 87.63**           | 290.98*           | 124.52**           | 72.82**           |
| 100         | 11.72**           | 8.63* | 17.76**        | 94.36**           | 313.64*           | 140.25**           | 86.39**           |
| Summer      |                   |    |                   |                   |                   |                   |                   |
| 0           | 1.94              | 7.64 | 0.51            | 25.68            | 165.88            | 17.86             | 23.69             |
| 20          | 3.72*             | 7.94 | 4.01*            | 35.69*            | 192.86*           | 31.75*            | 47.85*            |
| 40          | 6.36*             | 8.15* | 6.96**           | 60.25*            | 243.68*           | 75.84**           | 70.32**           |
| 60          | 8.48*             | 8.37* | 11.86**         | 81.36*           | 276.98*           | 114.56**           | 77.69**           |
| 80          | 10.55*            | 8.52* | 16.78**        | 89.69**           | 299.36*           | 138.69**           | 84.55**           |
| 100         | 12.89**           | 8.72* | 19.86**        | 97.47**           | 327.45*           | 156.87**           | 98.69**           |

Characteristics of soil

Physico-chemical characteristics of the soil changed due to irrigation with sugar mill effluent (Tables 2, 3, 4, 5, 6, 7, 8; Fig. 1). At harvest of *P. glaucum* (90 days after sowing), there was no significant change in the soil texture (loamy sand; 40 % sand; 40 % silt: 20 % clay). Irrigation with 100 % sugar mill effluent had the most increase in EC, OC, present study, the contents of BOD, COD, TKN, Cl\(^{-}\), SO\(_4^{2-}\), and PO\(_4^{3-}\) were more in sugar mill effluent than the content of BOD, COD, chlorides, sulfate, and phosphate in sugar mill effluent reported by Ezhilvannan et al. (2011). In the case of metals, the contents of Fe\(^{2+}\), Zn, Cd, Cu, Cr, and Mn were higher than permissible limits for industrial effluent (BIS 1991). The contents of these metals in sugar mill effluent were noted higher than the content of Zn, Cd, and Cu in the sugar mill effluent reported by Borole and Patil (2004).
Na⁺, K⁺, Ca²⁺, Mg²⁺, Fe²⁺, TKN, PO₄³⁻, SO₄²⁻, Cd, Cr, Cu, Mn, and Zn in both seasons (Tables 6, 7). The values of WHC and BD were insignificantly changed by the different concentrations of sugar mill effluent in both the cultivated seasons. WHC and BD were reduced from their initial (control) values 45.58 % and 1.42 gm cm⁻³ to 42.13, 41.36 %, and 1.41 gm cm⁻³, respectively, with 100 % sugar mill effluent concentration. Season, sugar mill effluent concentration, and interaction of seasons and sugar mill effluent concentration did not affect the WHC and BD (Table 2). WHC is related to the number and size

| Effluent/soil characteristics | Season | r value |
|-------------------------------|--------|---------|
| Sugar mill effluent versus soil WHC | Rainy | −0.97 |
|                                | Summer | −0.97 |
| Sugar mill effluent versus soil BD | Rainy | −0.96 |
|                                | Summer | −0.96 |
| Sugar mill effluent versus soil EC | Rainy | +0.98 |
|                                | Summer | +0.98 |
| Sugar mill effluent versus soil pH | Rainy | −0.96 |
|                                | Summer | −0.96 |
| Sugar mill effluent versus soil OC | Rainy | +0.99 |
|                                | Summer | +0.99 |
| Sugar mill effluent versus soil Na⁺ | Rainy | +0.97 |
|                                | Summer | +0.97 |
| Sugar mill effluent versus soil K⁺ | Rainy | +0.87 |
|                                | Summer | +0.87 |
| Sugar mill effluent versus soil Ca²⁺ | Rainy | +0.76 |
|                                | Summer | +0.76 |
| Sugar mill effluent versus soil Mg²⁺ | Rainy | +0.63 |
|                                | Summer | +0.63 |
| Sugar mill effluent versus soil TKN | Rainy | +0.99 |
|                                | Summer | +0.99 |
| Sugar mill effluent versus soil PO₄³⁻ | Rainy | +0.99 |
|                                | Summer | +0.99 |
| Sugar mill effluent versus soil SO₄²⁻ | Rainy | +0.96 |
|                                | Summer | +0.96 |
| Sugar mill effluent versus soil Fe²⁺ | Rainy | +0.99 |
|                                | Summer | +0.99 |
| Sugar mill effluent versus soil Zn | Rainy | +0.94 |
|                                | Summer | +0.94 |
| Sugar mill effluent versus soil Cd | Rainy | +0.97 |
|                                | Summer | +0.97 |
| Sugar mill effluent versus soil Cu | Rainy | +0.99 |
|                                | Summer | +0.99 |
| Sugar mill effluent versus soil Mn | Rainy | +0.99 |
|                                | Summer | +0.99 |
| Sugar mill effluent versus soil Cr | Rainy | +0.99 |
|                                | Summer | +0.99 |

Table 9 ANOVA for effect of sugar mill effluent on germination and vegetative growth of P. glaucum

| Source               | Seed germination | Relative toxicity | Plant height | Root length | No. of tillers | No. of leaves | Fresh weight | Dry weight |
|---------------------|-----------------|-------------------|--------------|-------------|----------------|---------------|---------------|------------|
| Season (S)          | ns              | ns                | ns           | ns          | ns             | ns            | ns            | ns         |
| SME concentration   | *               | ns                | *            | ns          | ns             | ns            | ns            | ns         |
| Interaction S × C   | *               | ns                | *            | ns          | ns             | ns            | ns            | ns         |

SME sugar mill effluent
ns, *, Non-significant or significant at P ≤ 0.05, ANOVA

Fig. 1 Contamination factor of heavy metals in soil after irrigation with sugar mill effluent. Error bars are standard error of the mean

Table 10 ANOVA for effect of sugar mill effluent on maturity stage of P. glaucum

| Source                     | Chlorophyll content | LAI | Spikes length | CY/plant | HI |
|----------------------------|---------------------|-----|---------------|----------|----|
| Season (S)                 | ns                  | ns  | ns            | ns       | ns |
| SME concentration (C)      | *                   | *   | *            | *        | ns |
| Interaction S × C          | *                   | *   | *            | *        | ns |

ns non-significant, SME sugar mill effluent

Table 8 Coefficient of correlation (r) between sugar mill effluent and soil characteristics in both seasons

| Effluent/soil characteristics | Season | r value |
|-------------------------------|--------|---------|
| Sugar mill effluent versus soil WHC | Rainy | −0.97 |
|                                | Summer | −0.97 |
| Sugar mill effluent versus soil BD | Rainy | −0.96 |
|                                | Summer | −0.96 |
| Sugar mill effluent versus soil EC | Rainy | +0.98 |
|                                | Summer | +0.98 |
| Sugar mill effluent versus soil pH | Rainy | −0.96 |
|                                | Summer | −0.96 |
| Sugar mill effluent versus soil OC | Rainy | +0.99 |
|                                | Summer | +0.99 |
| Sugar mill effluent versus soil Na⁺ | Rainy | +0.97 |
|                                | Summer | +0.97 |
| Sugar mill effluent versus soil K⁺ | Rainy | +0.87 |
|                                | Summer | +0.87 |
| Sugar mill effluent versus soil Ca²⁺ | Rainy | +0.76 |
|                                | Summer | +0.76 |
| Sugar mill effluent versus soil Mg²⁺ | Rainy | +0.63 |
|                                | Summer | +0.63 |
| Sugar mill effluent versus soil TKN | Rainy | +0.99 |
|                                | Summer | +0.99 |
| Sugar mill effluent versus soil PO₄³⁻ | Rainy | +0.99 |
|                                | Summer | +0.99 |
| Sugar mill effluent versus soil SO₄²⁻ | Rainy | +0.96 |
|                                | Summer | +0.96 |
| Sugar mill effluent versus soil Fe²⁺ | Rainy | +0.99 |
|                                | Summer | +0.99 |
| Sugar mill effluent versus soil Zn | Rainy | +0.94 |
|                                | Summer | +0.94 |
| Sugar mill effluent versus soil Cd | Rainy | +0.97 |
|                                | Summer | +0.97 |
| Sugar mill effluent versus soil Cu | Rainy | +0.99 |
|                                | Summer | +0.99 |
| Sugar mill effluent versus soil Mn | Rainy | +0.99 |
|                                | Summer | +0.99 |
| Sugar mill effluent versus soil Cr | Rainy | +0.99 |
|                                | Summer | +0.99 |
distribution of soil pores, soil moisture content, textural class, structure, salt content, and organic matter. The BD of soil changes with the application of organic manure to soil that substantially modifies, and lowers the soil bulk density. It is used for determining the amount of pore space and water storage capacity of the soil. Organic matter supplied through the sugar mill effluent and other kind of wastes, like sludge, can lower the BD and WHC (Roy et al. 2007). The findings were also in accordance to Baskaran et al. (2009). Fertigation with 100 % sugar mill effluent concentration increased pH (13.10–14.13 %), EC (510.41–564.43 %), OC (3,238.46–3,794.11 %), Na⁺ (270.32–279.55 %), K⁺ (90.44–97.40 %), Ca²⁺ (748.97–778.33 %), Mg²⁺ (268.71–316.58 %), TKN (1,083.73–1,121.84 %), PO₄³⁻ (287.47–301.10 %), SO₄²⁻ (239.20–244.45 %), Fe²⁺ (1,522.92–1,604.13 %), Cd (769.69–776.47 %), Cr (534.48–568.75 %), Cu (1,212.28–1,226.72 %), Mn (2,079.66–2,387.09 %), and Zn (1,707.14–2,317.54 %) in the soil in both seasons.

Sugar mill effluent concentration affected pH and EC of the soil but not season. Season, sugar mill effluent concentration and their interaction affected OC, and TKN of the soil (Tables 2, 4). The 40–100 % sugar mill effluent concentration affected EC, OC, Na⁺, K⁺, Ca²⁺, Mg²⁺, TKN, Fe²⁺, PO₄³⁻, SO₄²⁻, Cr, Cu, and Zn in P. glaucum cultivated soil in both seasons. The 20 % sugar mill effluent concentration also affected OC, Na⁺, Ca²⁺, Mg²⁺, TKN, PO₄³⁻, and SO₄²⁻ in both seasons (Tables 6, 7).

The EC, OC, Na⁺, K⁺, Ca²⁺, Mg²⁺, Fe²⁺, TKN, PO₄³⁻, SO₄²⁻, Cd, Cr, Cu, Mn, and Zn positively correlated with sugar mill effluent concentration in both seasons (Table 8). In the present study, more irrigation of P. glaucum with sugar mill effluent considerably increased the content of OC, Na⁺, K⁺, Ca²⁺, Mg²⁺, Fe²⁺, TKN, PO₄³⁻, SO₄²⁻, Cd, Cr, Cu, Mn, and Zn in soil. Season, sugar mill effluent concentration and their interaction affected all cations like Na⁺, K⁺, Ca²⁺, Mg²⁺, Fe²⁺ and anions PO₄³⁻ and SO₄²⁻ of the soil (Tables 3, 4).

Baskaran et al. (2009) reported that sugar mill effluent increased EC, pH, total organic carbon (TOC), total Kjeldahl nitrogen (TKN), and available phosphorus, exchangeable Na, K, Ca, and Mg in soil. Effluent irrigation generally adds PO₄³⁻, HCO₃⁻, Cl⁻, Na⁺, Ca²⁺, K⁺, Mg²⁺, Cd, Cr, Cu, Ni, Mn, and Zn to the soil (Chopra et al. 2009).

Total average organic matter content in the soil irrigated with effluent was higher than the soil irrigated with bore well water. The more organic matter in effluent irrigated soil might be due to the high organic nature of the effluent. Kumar and Chopra (2012) found the organic content in the soil irrigated with distillery effluent to be higher than in the soil irrigated with bore well water. Average values of TKN, PO₄³⁻, and K⁺ in the soil irrigated with effluent were found to be higher than in soil irrigated with bore well water. The high amount of TKN, PO₄³⁻, and K⁺ in the soil was due to irrigation with TKN, PO₄³⁻, and K⁺ rich sugar mill effluent. The content of Na⁺ and SO₄²⁻ was higher in the soil irrigated with sugar mill effluent indicating a link between soil Na⁺ and SO₄²⁻ and higher EC in the sugar mill effluent.

The contents of heavy metals, Cd, Cr, Cu, Mn, and Zn, in the soil increased as the effluent concentration increased (Table 7). Season, sugar mill effluent concentration, and their interaction affected all metals, Cd, Cr, Cu, Mn, and Zn in soil (Table 5). The concentration of Mn was found maximum while that of Cr was low after sugar mill effluent irrigation in both seasons. The contamination factor (Cf) of the heavy metals indicated that Mn was the highest while Cr was lower in both seasons after irrigation with 100 % sugar mill effluent. The Cf of heavy metals were in the

**Table 11** ANOVA for effect of sugar mill effluent on concentrations of metals in P. glaucum

| Source             | Zn     | Cd     | Cu     | Mn     | Cr     | Crude proteins | Crude fiber | Crude carbohydrates |
|--------------------|--------|--------|--------|--------|--------|----------------|-------------|---------------------|
| Season (S)         | *      | *      | *      | *      | *      | *              | *           | *                   |
| SME concentration (C) | **     | **     | **     | **     | **     | **             | **          | **                  |
| Interaction S × C  | **     | **     | **     | **     | **     | **             | **          | **                  |

SME sugar mill effluent

ns, *, ** Non-significant or significant at P ≤ 0.05 or P ≤ 0.01, ANOVA
order of Mn > Zn > Cu > Cd > Cr after irrigation with sugar mill effluent in both seasons (Fig. 1). The concentration of heavy metals Cd, Cr, Cu, Mn, and Zn was higher in soil irrigated with effluent than in soil irrigated with control water. Thus, fertigation with sugar mill effluent increased nutrients as well as metals content in soil.

Effect on germination

At 0–15 days after sowing, the best germination (95 and 93 %) was for with 40 % sugar mill effluent and the least (80 and 78 %) was due to treatment with 100 % sugar mill effluent (Fig. 2). Germination of P. glaucum was negatively correlated with sugar mill effluent concentrations in both seasons (Table 11). Seed germination of P. glaucum was affected by treatment (Table 9). The ANOVA indicated that season had no effect on plant germination and relative toxicity. Sugar mill effluent concentration and their interaction with season affected plant germination of P. glaucum, but not relative toxicity (Table 9).

The maximum RT (108.97 and 107.50 %) of sugar mill effluent against germination of P. glaucum was for the 100 % sugar mill effluent and it was positively correlated with sugar mill effluent concentrations in both seasons (Table 11, Fig. 3). The findings were very much in accordance with Radhouane (2008) reported that the germination of millet cultivars was decreased as concentration of the waste effluent increased from 0 to 100 %.

In the present investigation, the higher concentration of sugar mill effluent did not support plant germination. The higher concentration of sugar mill effluent lowered germination of P. glaucum likely due to the presence of high salt content in the effluent at these concentrations which inhibit germination. High concentrations are usually most damaging to young plants but not necessarily at germination, although the high salt concentration can slow germination by several days, or completely inhibit it. Because soluble salts move readily with water, evaporation moves salts to the soil surface where they accumulate and harden the soil surface delaying germination (Sunseri et al. 1998; Kaushik et al. 2004).

Effect on vegetative growth stage

Vegetative growth of P. glaucum at 45 days was affected in both seasons (Tables 9, 12). Maximum plant height, fresh weight, chlorophyll content, and LAI/plant of P. glaucum were due to treatment with the 40 % concentration of sugar mill effluent in both seasons (Table 12). Average root length (14.55 and 16.75 cm), number of tillers (4.67 and 5.96), number of leaves (14.88 and 16.36), and dry weight/plant (54.19 and 56.65 g) of P. glaucum were in control while root length (12.44 and 13.63 cm), number of tillers (3.21

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**Table 12** Effects of sugar mill concentration and season interaction on agronomical parameters of P. glaucum

| Season | SME % | Plant height (cm) | Fresh weight (g) | Chlorophyll content (mg (g f wt)) | LAI | Spike length (cm) | Kernels | Crop yield/plant (g) |
|--------|-------|-------------------|------------------|-----------------------------------|-----|------------------|---------|---------------------|
| Rainy  | 0     | 216.54            | 123.13           | 3.32                              | 2.35| 16.58            | 32.42   |
|        | 20    | 255.82*           | 143.56*          | 3.95ns                            | 3.55ns| 18.63ns          | 44.75ns |
|        | 40    | 314.29*           | 165.34*          | 4.81*                             | 5.19*| 28.58*           | 50.59*  |
|        | 60    | 265.84*           | 152.98*          | 4.71*                             | 4.76*| 25.36*           | 46.32*  |
|        | 80    | 234.78*           | 142.34*          | 4.42*                             | 4.43*| 22.48*           | 40.67ns |
|        | 100   | 225.36*           | 130.56 ns        | 3.45 ns                           | 3.81 ns| 20.18 ns         | 38.88 ns |
| Summer | 0     | 208.47            | 115.34           | 3.12                              | 2.12| 14.46            | 30.99   |
|        | 20    | 242.45*           | 137.56*          | 3.46ns                            | 3.46ns| 16.63ns          | 42.87ns |
|        | 40    | 301.23*           | 158.76*          | 4.62*                             | 5.12*| 27.44*           | 48.89*  |
|        | 60    | 255.48*           | 147.23*          | 4.29*                             | 4.29*| 24.66*           | 42.98ns |
|        | 80    | 225.32*           | 135.55*          | 4.18*                             | 4.37*| 20.75*           | 38.78ns |
|        | 100   | 217.88*           | 124.78ns         | 3.38ns                            | 3.68ns| 18.56ns          | 36.89ns |

*SME* sugar mill effluent

* Significant at $P \leq 0.05$, Least Means Squares analysis
and 3.55), number of leaves (12.55 and 13.75), and dry weight/plant (43.89 and 44.38 g) of *P. glaucum* were with 100 % sugar mill effluent in both seasons. The maximum root length (18.36 and 20.36 cm), number of tillers (7.45 and 7.89), number of leaves (18.87 and 18.96), and dry weight/plant (61.36 and 62.87 g) of *P. glaucum* were with 40 % concentration of sugar mill effluent in both seasons. Sugar mill effluent concentration, season, and their interaction had no effect on root length, number of tillers, number of leaves, and dry weight of *P. glaucum* (Tables 9, 10).

The ANOVA indicated that the sugar mill effluent concentration affected plant height, fresh weight, chlorophyll content, and LAI/plant of *P. glaucum* (Tables 9, 10). The season had no effect on plant height, fresh weight, chlorophyll content, and LAI/plant of *P. glaucum*. The interaction of the season and sugar mill effluent concentrations affected plant height, fresh weight, chlorophyll content, and LAI/plant of *P. glaucum* (Tables 9, 10).

Plant height, number of tillers, number of leaves, fresh weight, dry weight, chlorophyll content, and LAI/plant of *P. glaucum* were positively correlated with sugar mill effluent concentrations in both seasons (Table 11). Root length was positively correlated with sugar mill effluent concentrations in the rainy season while it was negatively correlated in the summer season (Table 11). The findings were in accordance with Moazzam et al. (2012).

Vegetative growth of *P. glaucum* was lowered at higher concentrations of sugar mill effluent. A high EC indicates higher salt content in the higher sugar mill effluent concentrations, which lowered the plant height, root length, number of tillers, number of leaves, fresh weight, dry weight, chlorophyll content, and LAI/plant of *P. glaucum*. Vegetative growth is associated with the development of new shoots, twigs, leaves, and leaf area. Plant height, fresh weight, chlorophyll content, and LAI/plant of *P. glaucum* were higher at 40 % of sugar mill effluent; it may be due to maximum uptake of nitrogen, phosphorus, and potassium by plants. The improvement of vegetative growth may be attributed to the role of potassium in nutrient and sugar translocation in plants and turgor pressure in plant cells (Al-Tahir et al. 1997). It is also involved in cell enlargement and in triggering young tissue or mersitematic growth (Arya et al. 1997; Radhouane 2008). Chlorophyll content was higher due to the use of 40 % sugar mill effluent in both seasons, and it is likely due to Fe, Mg, and Mn contents in the sugar mill effluent, which are associated with chlorophyll synthesis (Porra 2002). The 40 % sugar mill effluent concentration contains optimum contents of nutrients required for maximum vegetative growth of *P. glaucum*.

Effect on maturity stage

On maturity stage (90 days after sowing), the spikes length and crop yield/plant of *P. glaucum* was with 40 % sugar mill effluent in both seasons (Table 12). Spikes length and crop yield of *P. glaucum* decreased as the sugar mill effluent concentration decreased (Table 12). Maximum HI (156.04 and 157.76 %) was with 40 % sugar mill effluent concentration and (119.03 and 119.92 %) with 100 % sugar mill effluent in both seasons. Sugar mill effluent concentration affected spikes length and crop yield of *P. glaucum* but season, interaction of the season and sugar mill effluent concentration did not have an effect on spikes length, crop yield of *P. glaucum* (Table 10). Season and sugar mill effluent concentration had no effect on Harvest

| Effluent/French bean | Season  | r value |
|----------------------|---------|---------|
| Sugar mill effluent versus seed germination | Rainy | −0.53 |
| Sugar mill effluent versus RT | Rainy | +0.76 |
| Sugar mill effluent versus plant height | Rainy | +0.10 |
| Sugar mill effluent versus root length | Rainy | +0.13 |
| Sugar mill effluent versus number of tillers | Rainy | +0.56 |
| Sugar mill effluent versus number of leaves | Rainy | +0.58 |
| Sugar mill effluent versus fresh weight | Rainy | +0.07 |
| Sugar mill effluent versus dry weight | Rainy | +0.05 |
| Sugar mill effluent versus chlorophyll content | Rainy | +0.16 |
| Sugar mill effluent versus LAI | Rainy | +0.50 |
| Sugar mill effluent versus spikes length | Rainy | +0.32 |
| Sugar mill effluent versus crop yield/plant | Rainy | +0.05 |
| Sugar mill effluent versus HI | Rainy | +0.10 |
| Sugar mill effluent versus Zn | Rainy | +0.95 |
| Sugar mill effluent versus Cd | Rainy | +0.86 |
| Sugar mill effluent versus Cu | Rainy | +0.99 |
| Sugar mill effluent versus Mn | Rainy | +0.99 |
| Sugar mill effluent versus Cr | Rainy | +0.89 |
| Sugar mill effluent versus Cr | Summer | +0.90 |
index (HI) of *P. glaucum*. Crop yield of *P. glaucum* was positively correlated with sugar mill effluent concentrations in the rainy season while it was negatively correlated in the summer season (Table 11). The results were supported by Zalawadia and Raman (1994).

Nitrogen and phosphorus are essential for flowering and spike length. Too much nitrogen can delay, or prevent, flowering while phosphorus deficiency is sometimes associated with poor flower production, or flower abortion. Maximum spike length of *P. glaucum* was with the 40 % sugar mill effluent; it might be due to that this concentration contains sufficient nitrogen and phosphorus. Furthermore, P and K prevent flower abortion, so grain formation occurs (El-Naggar 2005). Spikes length of *P. glaucum* was lower at higher concentrations of sugar mill effluent. This is likely due to increased content of metals in the soil, which inhibits uptake of P and K by plants at higher sugar mill effluent concentrations (Pandey et al. 2008). The role of K, Fe, Mg, and Mn at maturity is important and associated with synthesis of chlorophyll, and enhances the formation of grains at harvest (El-Naggar 2005; Naeem et al. 2006). The K, Fe, Mg, and Mn contents could benefit grain filling and yield as it does for pearl millet (*P. glaucum L.*). The 40 % sugar mill effluent favored grains formation and crop yield of *P. glaucum*. This is likely due to the presence of K, Fe, Mg, and Mn contents in 40 % sugar mill effluent; higher sugar mill effluent concentrations lowered grains formation and crop yield of *P. glaucum*.

Effect on biochemical constituents and micronutrients in *P. glaucum*

Season, sugar mill effluent concentration, and the interaction of the season and sugar mill effluent concentration affected all the metals like crude proteins, crude fiber, and crude carbohydrates, Cd, Cr, Cu, Mn and Zn in *P. glaucum* (Table 13). Maximum crude proteins, crude fiber, and crude carbohydrates were recorded with 40 % sugar mill effluent concentrations in both seasons (Figs. 4, 5, 6). Content of crude proteins (*r* = + 0.38), crude fiber (*r* = + 0.17) and crude carbohydrates (*r* = + 0.13) was noted positively correlated with sugar mill effluent concentration in both seasons. The 20, 40, 60, 80, and 100 % sugar mill effluent concentrations affected Cd, Cr, Cu, Mn, and Zn contents in *P. glaucum* (Table 13). Increased irrigation frequency could lead to increases of metals in tissues. The Cd, Cr, Cu, Mn, and Zn contents in *P. glaucum* was the highest with 100 % sugar mill effluent (Figs. 7, 8). They were positively correlated with content of Cd, Cr, Cu, Mn, and Zn in *P. glaucum* after irrigation with sugar mill effluent concentrations in both seasons (Table 11). The contamination factor (Cf) was affected in both seasons (Fig. 9). The Cf of various heavy metals was in the order of Mn > Zn > Cu > Cr > Cd in *P. glaucum* after irrigation with sugar mill effluent (Fig. 9). The highest contamination factor was for Mn; the least was for Cd in *P. glaucum* with 100 % sugar mill effluent in both seasons. The micronutrient contents were higher at higher sugar mill

![Fig. 4](image-url) Crude proteins in *P. glaucum* after irrigation with sugar mill effluent. Error bars are standard error of the mean

![Fig. 5](image-url) Crude fiber in *P. glaucum* after irrigation with sugar mill effluent. Error bars are standard error of the mean

![Fig. 6](image-url) Crude carbohydrates in *P. glaucum* after irrigation with sugar mill effluent. Error bars are standard error of the mean
effluent concentration, and likely inhibited growth of *P. glaucum*. The 40% sugar mill effluent favored vegetative growth, flowering, and maturity of *P. glaucum*. This is likely due to optimal uptake of these micronutrients by crop plants, which supports various biochemical and physiological processes.

**Conclusions**

The present study concluded that the sugar mill effluent increased nutrients in the soil and affected the agronomical characteristics of *P. glaucum* in both seasons. The maximum agronomical performance of *P. glaucum* was observed with 40% concentration of the sugar mill effluent concentration.
effluent. It appears that sugar mill effluent can be used as a biofertilizer after appropriate dilution to improve yield of this crop. Further studies on the agronomic growth and changes in biochemical composition of *P. glaucum* after sugar mill effluent irrigation are required.

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