



Effect of brewery wastewater obtained from different phases of treatment plant on seed germination of chickpea (*Cicer arietinum*), maize (*Zea mays*), and pigeon pea (*Cajanus cajan*)

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Abstract

Brewing industry releases large quantities of wastewater after product generation. Brewery wastewater contains organic compounds which are biodegradable in nature. These biodegradable wastes can be recycled and reused and hence considered as suitable products for agriculture. But before using wastewater for agriculture, it is better to evaluate the phytotoxic effects of wastewater on crops. Hence, the main objective of this study is to evaluate the effects of brewery effluent on seed germination and growth parameters of selected crop species like chickpea (*Cicer arietinum*), maize (*Zea mays*), and pigeon pea (*Cajanus cajan*). Study comprised seven types of water treatments—tap water as control, diluted UASBR effluent (50% effluent + 50% distilled water): UASBR50, undiluted UASBR effluent: UASBR100, diluted TC effluent (50% effluent + 50% distilled water): ETP50, TC effluent without dilution: ETP100, 10% diluted reverse osmosis (RO10) reject (10% RO reject + 90% distilled water), and 25% diluted reverse osmosis (RO25) reject (25% RO reject + 75% distilled water) with three replications in completely randomized design. Germination test was performed in petri plates for 5 days. Parameters like germination percentage, germination rate index, seedling length, phytotoxicity index, seed vigor index, and biomass were calculated. All parameters decreased with increase in respective effluent concentration. Among all treatments, RO25 showed highest inhibitory effect on all three crops. Even though undiluted effluent of UASBR and ETP effluent showed positive effect on germination, seedling growth of three crops was promoted to the maximum by UASBR50 and ETP50. Hence, from the study, it was concluded that dilution of brewery effluent can be recommended before using it for irrigational purpose.

Keywords Brewery wastewater · Germination · Chickpea · Maize · Pigeon pea

Introduction

Water plays an important role in food production from agriculture and product generation from industrial activities. Water is scarce natural resource and agriculture is large user of water for irrigation purposes. Very less percentage of water sources are available for industries and hence industrial sector

is competing for water resources with agriculture and domestic sector. Industrial development leads to employment generation but unintentionally by releasing wastewater, it may pollute the water bodies and soil complex systems (Panasker and Pawar 2011). Hence, if this wastewater generated from industries could be recycled and reused in environmental friendly manner, it may solve water scarcity and pollution problems.

Brewing industry releases large quantities of wastewater, nearly 3 to 10 l of wastewater per liter of beer produced (Genner 1988). Brewery industry involves production of beer using cereal grains by two main processes, namely, malting and brewing. During the beer preparation, wastewater is mainly generated from pressing wet grain and other source includes wash water from different departments of industry (Noorjahan and Jamuna 2012). In specific, beer production utilizes two thirds of water and rest one third is used for washing, cleaning, and packing (Moll and Bieres 1991). As there are different

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processes involved in beer production, the quality of effluent discharged varies considerably. Treatment of brewery wastewater generally depends on socio-economic, environmental parameters and also on quality of wastewater generated.

Brewery wastewater generally contains high organic load and for treating such effluent, high energy inputs are required for aerobic treatment. Aerobic treatment also leads to high quantity of sludge generation and disposal of sludge poses immense problems. Hence, pre-treatment of high organic loaded brewery wastewater with anaerobic processes followed by aerobic treatment forms suitable solution for above said problems (Rodrigues et al. 2001). Various types of anaerobic reactors are available for brewery effluent treatment, but among them, upflow anaerobic sludge blanket reactor was found to be the most usual full-scale system (Parawira et al. 2005). To obtain the required standards for discharge into water stream, advanced treatment techniques like use of multigrade filters and filtration techniques using ultra filtration/Reverse Osmosis after aerobic treatment may be employed. Each treatment process employed will have its specific pollutant removing/degrading capability. Anaerobic treatment is effective in removing COD (Shao et al. 2008; Leal et al. 1998; Chaitanyakumar et al. 2011) and BOD. But anaerobic treatment was found to be ineffective in reducing TSS and TDS levels. Aerobic process is effective on COD, BOD, and TSS removal and inefficient for TDS removal. One hundred percent TSS removal can be achieved through Ultra filtration unit; Reverse Osmosis shows marked reduction of TDS removal and also, COD and BOD reduction of brewery wastewater (Chaitanyakumar et al. 2011).

Brewery effluent contains organic compounds like sugars, starch, ethanol etc., which are biodegradable in nature (Driessen and Vereijken 2003). Solid by-products generated from breweries include spent grains, hops, and yeast. These biodegradable and solid wastes are recycled and reused and hence considered as suitable products for agriculture. This feature of brewery effluent makes it more eco-friendly for reuse options when compared to other industrial effluents (Ishiwaki et al. 2000). But brewery effluent contains high organic content which leads to higher BOD and COD concentrations. This effluent rich in organic content if lead into water course may damage lake ecology, thereby affecting all forms of life (ChaitanyaKumar et al. 2011).

As there are serious environmental concerns and strict regulations, attempts are being made in developing countries like India to utilize brewery wastes in eco-friendly manner (Senthilraja et al. 2013). Stringent norms and sound policies are required to curb the pollution of water bodies by industrial effluents and also to safeguard the environment. The only viable option left for water scarcity and pollution problems is safe reuse of wastewater. Hence, present study aims to focus on reuse options of brewery effluent for agriculture. For this purpose, seed germination bioassay was conducted to evaluate

the effects of brewery effluent on germination and growth parameters of selected crop species like chickpea, maize, and pigeon pea.

Materials and methods

Brewery wastewater collection and characterization

Brewery wastewater samples were collected from beer factory located near Sangareddy, Telangana (India). Brewery wastewater treatment employed at factory is a multistage process and contains many phases of treatment. Treatment process included anaerobic treatment with upflow anaerobic sludge blanket reactor (UASBR) followed by aerobic treatment by activated sludge process in two stages. Aerobically treated water from activated sludge process flows into primary clarifier from where it will again undergo treatment in another activated sludge unit. Water treated in second unit of activated sludge process will flow into secondary and then to tertiary clarifier (TC). Water from TC will be passed through different types of filters like multigrade and activated carbon filter. Finally, the treated water from filters is allowed to pass through ultrafiltration unit followed by reverse osmosis. Each phase of treatment has immense importance in reducing specific type of pollutants.

Wastewater samples were collected from effluent treatment plant of factory at three different points, i.e., (1) effluent of upflow anaerobic sludge blanket reactor (UASBR), (2) effluent of tertiary clarifier (ETP), and (3) reject effluent of reverse osmosis plant (RO). Collected samples were analyzed for physico-chemical parameters using standard methods (APHA 2005).

Germination bioassay experimental

Study comprised seven types of water sources—tap water as control, diluted UASBR effluent (50% effluent + 50% distilled water): UASBR50, undiluted UASBR effluent: UASBR100, diluted TC effluent (50% effluent + 50% distilled water): ETP50, TC effluent without dilution: ETP100, 10% diluted reverse osmosis (RO10) reject (10% RO reject + 90% distilled water), and 25% diluted reverse osmosis (RO25) reject (25% RO reject + 75% distilled water) with three replications in completely randomized design. Seeds of selected crops like chickpea (cultivar: JG-11), maize (cultivar: HTMH 5404), and pigeon pea (cultivar: ICPL-88039) were sterilized by ethyl alcohol. Ten seeds of each crop were placed in sterilized petri plate lined with filter paper. Five milliliter of wastewater was added in each petri plate and then incubated at room temperature. Following parameters were evaluated after every 24 h up to 5-day period.

- (a) Germination percentage—number of germinated seeds in each petri plate was counted after 24 h and germination percentage was calculated using following formula (Naeem et al. 2015)

$$\text{Germination}\% = \frac{\text{Number of seeds germinated} \times 100}{\text{Total number of seeds}}$$

- (b) Germination rate index (GRI) was calculated using the following equation (Wang et al. 2004).

$$Gt = \sum(Gt/Tt)$$

where,

Gt Germination percentage at *t*th day

Tt Day of germination test

Higher value of GRI indicates rapid rate of germination and exhibits quality and better performance of seed (Wang et al. 2004).

- (c) Seedling length—after 5-day period, germinated seeds of each crop were taken and their radical and plumule lengths were measured using a scale. Plumule length measurement was taken from base of hypocotyl to primary leaf. Similarly, radical length was measured from primary root tip to hypocotyl base. Both radical and plumule length were expressed in centimeters. Finally, seedling length in centimeters was obtained by adding both radical and plumule length (Dash 2012).

$$\text{Seedling length (cm)} = \text{Radical length (cm)} + \text{Plumule length (cm)}$$

- (d) Phytotoxicity index (PI)—PI was calculated based on germination and root length using following equation (Rusan et al. 2015)

$$PI = 1 - \frac{RLT}{RLC}$$

where,

RLT Root length of treated seeds

RLC Root length of control seeds

- (e) Seed vigor index—calculated by using following formula (Abdul-Baki and Anderson 1973).

$$SVI = \text{Germination}\% \times \text{Seedling length}$$

where,

SVI Seed vigor index

- (f) Biomass—seedlings were oven dried at 60 °C for 48 h and their biomass (dry weight) was determined using weighing balance (Aguirre and Johnson 1991)

Statistical analysis

The data obtained was subjected to statistical analysis using ANOVA (Analysis of Variance). If the calculated *F* ratio was significant, then Fisher's least significance test at 0.10 probability level was performed for multiple mean comparisons.

Results and discussion

Brewery wastewater characteristics

Physico-chemical parameters of wastewater collected from different phases of brewery industry treatment plant are represented in Table 1 below.

pH of brewery wastewater collected from different phases of treatment plant was almost similar to tap water, but pH of ETP effluent was slightly higher (7.83). pH of brewery effluent mainly depends on the quantity and type of chemicals used in cleaning unit like phosphoric acid, nitric acid, and caustic soda. Highest value of EC was recorded with RO reject outlet (12.07 mS) followed by UASBR and ETP outlet (4.37 and 4.04 mS) respectively. Lowest EC value was observed with tap water (0.62 mS). TDS concentration was highest for RO reject outlet (7578 mg l⁻¹) and lowest TDS value was observed in tap water (445 mg l⁻¹). Highest TSS value was recorded for UASBR outlet effluent (46.5 mg l⁻¹) and lowest value for tap water (5.06 mg l⁻¹). High concentration of total solids may be due to carbonates, chlorides, sulfates, and nitrates of Ca, Mg, and Na that leads to high salinity in water (Pandey and Sharma 2002). Important plant nutrient like nitrates was highest for ETP outlet effluent (20.46 mg l⁻¹) followed by RO reject outlet (16.29 mg l⁻¹) respectively and lowest value was observed in tap water (1.82 mg l⁻¹). Highest value of ammonical nitrogen (NH₄-N) was noted for UASBR outlet effluent (66.82 mg l⁻¹) among all water types and lowest value was recorded with RO reject outlet and tap water (10.06 and 2.67 mg l⁻¹) respectively. Nitrogen concentration in the brewery effluent depends upon the raw material handling and quantity of spent yeast (Parawira et al. 2005). Phosphate concentration was very low in tap water (0.02 mg l⁻¹) and highest value was recorded for UASBR outlet (1.57 mg l⁻¹). Parameters like potassium, chlorides, COD, fluorides, sodium, total

Table 1 Physico-chemical parameters of tap water and brewery effluent (mean \pm SE)

Parameter	Tap water	UASBR outlet	ETP outlet	RO reject outlet
pH	7.47 \pm 0.1	7.37 \pm 0.1	7.83 \pm 0.2	6.93 \pm 0.3
EC ^a (ms/cm)	0.62 \pm 0.06	4.37 \pm 0.54	4.04 \pm 0.40	12.07 \pm 1.64
TDS ^b (mg/l)	445.6 \pm 9	2737.22 \pm 256.8	2556.56 \pm 214.7	7578.50 \pm 1238.6
TSS ^c (mg/l)	5.06 \pm 0.5	46.50 \pm 8.8	5.50 \pm 0.6	27.00 \pm 6.3
Boron (mg/l)	0.063 \pm 0.01	0.09 \pm 0.01	0.08 \pm 0.01	0.16 \pm 0.02
Calcium (mg/l)	43.89 \pm 4.18	40.24 \pm 6.28	39.50 \pm 4.03	93.64 \pm 14.54
Chlorides (mg/l)	53.88 \pm 7.04	244.28 \pm 23.46	229.82 \pm 23.99	430.15 \pm 81.98
COD ^d (mg/l)	49 \pm 12.58	350 \pm 30.22	202.50 \pm 55.83	365.33 \pm 120.2
Detergents (mg/l)	—	0.662 \pm 0.02	0.55 \pm 0.01	0.88 \pm 0.0
Flourides (mg/l)	1.27 \pm 0.14	1.88 \pm 0.16	1.78 \pm 0.16	2.13 \pm 0.21
Iron (mg/l)	0.14 \pm 0.07	1.04 \pm 0.17	0.43 \pm 0.14	1.56 \pm 0.49
Magnesium (mg/l)	27.05 \pm 1.11	28.24 \pm 1.44	26.57 \pm 2.62	67.77 \pm 7.15
Ammonical nitrogen (mg/l)	2.67 \pm 0.73	66.82 \pm 12.21	11.13 \pm 2.73	10.06 \pm 3.85
Nitrate nitrogen (mg/l)	1.82 \pm 0.5	7.35 \pm 0.9	20.46 \pm 6.5	16.29 \pm 1.18
Phosphates (mg/l)	0.02 \pm 0.0	1.57 \pm 0.4	1.25 \pm 0.2	0.76 \pm 0.3
Potassium (mg/l)	14.87 \pm 3.7	50 \pm 4.5	47.20 \pm 3.3	119.05 \pm 21.4
Sodium (mg/l)	12.00 \pm 0.1	527 \pm 100	416.28 \pm 27.05	1126.77 \pm 529.4
Total alkalinity (mg/l)	128 \pm 20.13	731 \pm 47.7	551.2 \pm 29.3	1138 \pm 322.6
Total hardness (mg/l)	134.6 \pm 17.3	305.7 \pm 22.3	281 \pm 16.8	685 \pm 83.7

^a Electrical conductivity^b Total dissolved solids^c Total suspended solids^d Chemical oxygen demand

Heavy metals like arsenic, cadmium, chromium, cobalt, lead, nickel, and zinc are below detectable limit (BDL) in above water sources

hardness, and alkalinity were highest in RO reject outlet (119.05, 430, 365.33, 2.13, 1126.77¹, 685, and 1138 mg l⁻¹ CaCO₃) respectively and lowest values were recorded in tap water (14.87, 53.88, 49, 1.27, 12, 134.66, and 128 mg l⁻¹ CaCO₃) respectively. Calcium and magnesium concentrations were also highest with RO reject outlet (93.64 and 67.77 mg l⁻¹) and lowest concentrations were observed in ETP outlet effluent (39.50 and 26.57 mg l⁻¹) respectively. Heavy metals like boron and iron were highest in RO reject outlet effluent (0.16 and 1.56 mg l⁻¹) respectively. Remaining heavy metals are in below detectable range in all water sources.

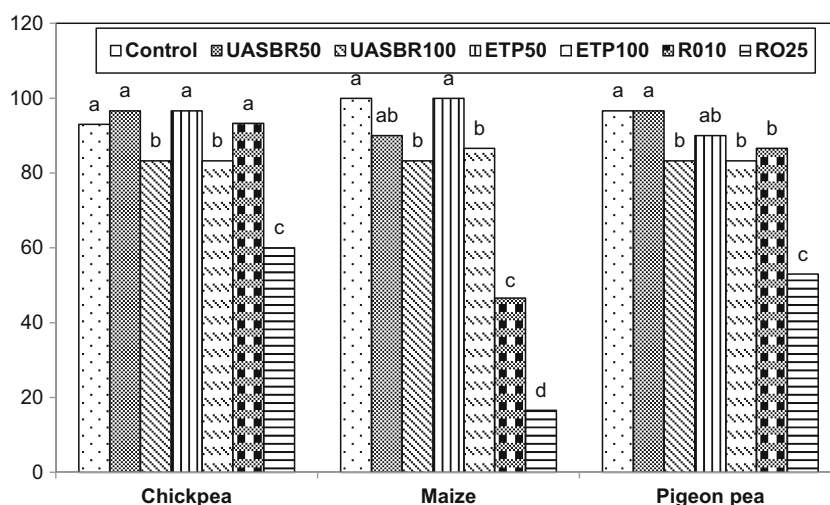
Germination percentage

Germination percentage of tested crops is represented in Fig. 1.

Percentage of seed germination decreased gradually with increase in effluent concentration in all three crops. Other investigators also found that germination decreased with increased effluent concentration (Reddy and Borse 2001, Ramana et al. 2002, Mensuh et al. 2006, Pandey et al. 2007, Sandeep et al. 2007, Anupama 2011, Malaviya and Sharma 2011, Asfi et al. 2012, Narain et al. 2012a, b, Naeem et al.

2015, Abu-Dieyeh et al. 2017). In chickpea, 97% seed germination was recorded for both UASBR50 and ETP50. Germination percentage for RO10 was similar to that of control (tap water) with 93% germination. But, RO25 showed inhibitory effect with higher salt concentration on germination as indicated by very less germination (60%). Even UASBR100 and ETP100 showed positive effect with 83% seed germination. In maize, 100% seed germination was recorded with both ETP50 and control, whereas 90% germination was observed with UASBR50. UASBR100 and ETP100 also showed positive effect with 83 and 87% germination, respectively. Both dilutions of RO reject showed very less germination percentage, i.e., 47% with RO10 and 17% with RO25. In pigeon pea, 97% seed germination was recorded for control and UASBR50 followed by 90% for ETP50. RO reject outlet at 10% dilution exhibited 87% germination followed by 83% with UASBR100 and ETP100. A negative effect of higher salt content on seed germination was also observed for pigeon pea as germination percentage in RO25 was only 53%. Similar inhibitory effects of higher salt concentrations were expressed by Barazani and Golan-Goldhirsh (2009) on germination of *Salsola inermis* and by Khan et al. (2017) on rice germination. Osmotic pressure and ion toxicity of salts present in brewery wastewater (RO reject) may be the reason for

Fig. 1 Effect of brewery wastewater on seed germination of chickpea, maize, and pigeon pea. Means with the same letter are not significantly different from one another at 10% level of probability within different treatments



adverse effects on seed germination as stated by Rusan et al. (2015) on barley germination by application of olive mill wastewater. Osmotic pressure due to higher salt concentration mainly shows effect on water uptake by seeds thus leading to inhibition/reduction of seed germination (Naeem et al. 2015).

Among all three crops, maize was affected the most by RO reject outlet effluent as least values of germination percentage were observed in maize as when compared to other two crops. Depending on tolerance levels to RO reject effluent, crop species can be arranged as maize > pigeon pea > chickpea. Ramana et al. (2002) and Abu-Dieyeh et al. (2017) inferred similar tolerance levels of crop species to distillery effluent and bakery yeast wastewater, they arranged crops in the following manner: cucumber > chili > onion > bottle gourd > tomato and tomato > barley > squash respectively. Similarly, studies revealed that maize was more sensitive to distillery effluent than rice (Pandey et al. 2008) and lady's finger than pea (Sandeep et al. 2007), whereas tomato was more sensitive to raw olive mill wastewater than pea (Mseddi et al. 2016).

Promoting effect on seed germination with diluted UASBR and ETP effluents can be attributed to more nutrients present in the wastewater than tap water as shown in Table 1. Wastewater contains some essential organic compounds which promote growth of crops (Pathak et al. 1999; Ramana et al. 2002; Nagajyothi et al. 2009). But, at higher concentrations of these effluents (UASBR100 and ETP100), germination percentage decreased indicating that higher concentrations of effluent may pose toxic effect (Nagda et al. 2006; Khan et al. 2011). Similar results have been inferred in maize by using textile and dyeing industrial effluents (Mathur et al. 1997), in *C. argentea* using brewery wastewater (Ogunwenmo et al. 2010), in sorghum by using textile mill effluent (Panasker and Pawar 2011), in chickpea using distillery effluent (Narain et al. 2012a), and in rice using municipal wastewater (Gassama et al. 2015).

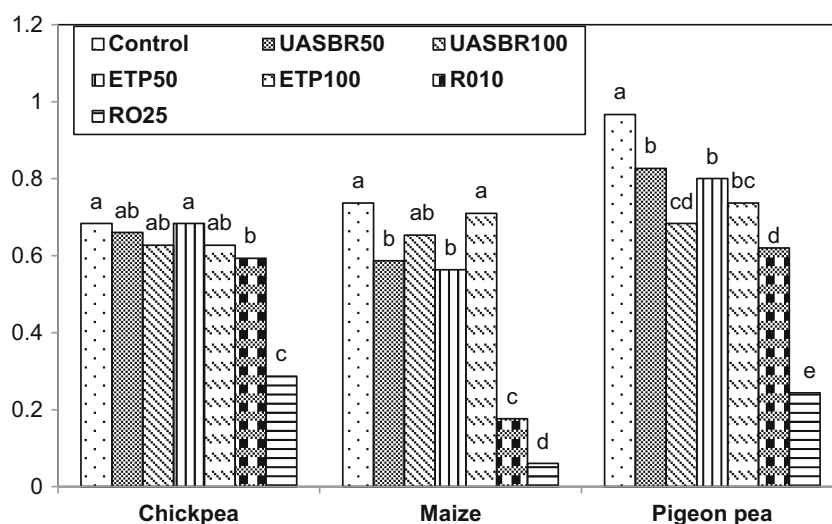
In all three tested crops, RO25 showed inhibitory effect on seed germination. This can be owed to higher EC and TDS values

of RO reject outlet, represented in Table 1. High concentration of dissolved solids increases the salinity leading to inhibition of seed germination (Gautham and Bishoni 1992). Abu-Dieyeh et al. (2017) stated that higher level of salinity caused by soluble salts and chlorides is associated with elevated osmotic pressure of the medium which will lead to reduction in seed germination. They also inferred that organic and inorganic elements occurring in wastewater might also be injurious to seed germination. Ramana et al. (2002) found that inhibition in germination might be due to higher concentrations of inorganic salts and higher EC values of the distillery effluent. Similarly, Singh et al. (2002) found that germination of *Albizialebeck* decreased due to high pH and EC of textile and dyeing industries effluents as irrigation water. Whereas, household waste effluent contained high values of conductivity, turbidity, orthophosphates, COD, and salt (NaCl) which were reported to have strongly inhibited seed germination of wheat (Djelal et al. 2014). Thus, from studies of various authors, it can be observed that magnitude of toxicity of effluent depends on nature and concentration of chemicals present in it and hence inhibition/reduction of seed germination cannot be attributed to single factor.

Germination rate index

In chickpea, highest GRI (0.68) was recorded with ETP50, which was similar to control. Lowest GRI (0.29) in chickpea was observed with RO25. In maize, highest GRI (0.74) was recorded with control and ETP100 (0.71), whereas lowest GRI (0.06) was recorded with RO25. In pigeon pea, highest GRI (0.97) was recorded with control and UASBR50 (0.83); similar to chickpea and maize, lowest GRI (0.24) was recorded with RO25. Among all crops, highest GRI value was observed in pigeon pea for UASBR50 (0.83) and lowest GRI (0.06) for RO25 in maize (Fig. 2). Lowest GRI in all crops with RO reject effluent can be attributed to high dissolved salts. Naseri et al. (2012) and Rusan et al. (2015) also encountered similar impact of salinity on GRI of barley seeds.

Fig. 2 Effect of brewery wastewater on germination rate index of chickpea, maize, and pigeon pea. Means with the same letter are not significantly different from one another at 10% level of probability within different treatments



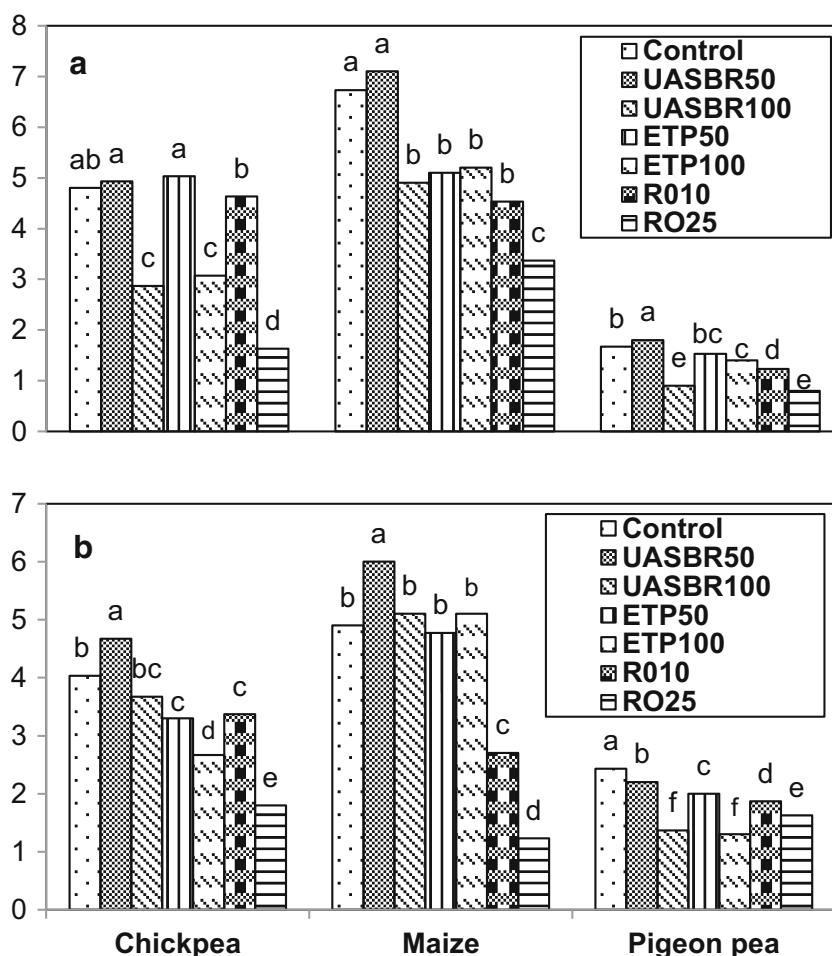
Seedling length

Radical and plumule length also exhibited declining trend from lowest to highest concentrations of effluent in all three crops as shown in Fig. 3. Similar results of declining trend of radical and plumule lengths with respect to effluent concentration was inferred

by Anupama (2011), Yasmin et al. (2011), Narain et al. (2012a, b), and Zhang et al. (2015). But in maize, ETP50 and ETP100 showed similar promoting effect on radical and plumule length.

Radical length in chickpea was highest with ETP50 (5.03 cm), whereas in maize and pigeon pea, highest radical length of 7.1 and 1.8 cm, respectively, was observed with

Fig. 3 Effect of brewery wastewater on **a** radical length (cm) and **b** plumule length (cm) of tested crops. Means with the same letter are not significantly different from one another at 10% level of probability within different treatments



UASBR50. Lowest value of radical length in all three tested crops was recorded with RO25, i.e., in chickpea (1.63 cm), maize (3.37 cm), and in pigeon pea (0.8 cm). Similarly, in mung bean (Augusthy and Sherin 2001) and rice seedlings (Gassama et al. 2015), root length increased at lower concentrations when compared to high concentrations of wastewater. Rani and Srivastava (1990) observed that distillery effluent at higher concentrations inhibited the root growth in *Pisum sativum* as it contains high levels of BOD and soluble salts, whereas Abu-Dieyeh et al. (2017) concluded that decrease in seedling lengths of tomato was due to elevated total dissolved solids in the bakery yeast wastewater. Increased salinity stress of effluents will affect the water uptake by seeds, thereby slowing down the water uptake and eventually leading to inhibition of germination and decreased root elongation (Naeem et al. 2015).

In chickpea and maize, highest plumule length of 4.67 and 6 cm, respectively, were observed with UASBR50. But in pigeon pea, highest plumule length was recorded with control (2.43 cm). Similar to that of radical length, plumule length was also lowest with RO25 in two crops. Lowest plumule length values of 1.8 cm in chickpea and 1.23 cm in maize were recorded. In pigeon pea, lowest plumule length values of 1.3 cm with UASBR100 and 1.37 cm with ETP100 were observed. Bazai and Achakzai (2006) also found that domestic wastewater has promoting effect on plumule length of lettuce at lower concentrations, but at higher concentrations, it leads to inhibitory effect. In pigeon pea, elevated concentrations of effluent showed negative impact on radical length than plumule length. This might be due to long contact of roots with effluent which allowed phytotoxic substances to affect root structure, metabolic efficiency, and its stability, thus leading to decreased radical lengths (Asfi et al. 2012).

Stimulatory effect of lower concentrations of effluents can be attributed to nutrients present in them which were available to seedlings and hence there was increase in growth of seedlings (Naeem et al. 2015). Wastewater contains different types of nutrients and their positive or negative effect depends on

the elemental concentration and also, their impacts cannot be attributed to single element. This statement was in line with Yousaf et al. (2010) who stated that promoting effect of municipal wastewater on seedling length was due to presence of nitrates and sulfates which stimulate plant growth by proteins and other beneficial organic molecule production (Yousaf et al. 2010). da Costa Marques et al. (2015) expressed that synthetic oilfield produced has lesser amounts of organic compounds and also contained micronutrients like Zn, Mn, and Fe which might have contributed to stimulatory effect on sunflower seedling growth. But Lafragueta et al. (2014) concluded that aqueous extract of cement dust was found to have high concentrations of calcium, potassium, sodium, sulfur, and chromium and so alfalfa germination was not affected by individual element, but it was synergistic effect of mixture of all elements, whereas Lauchli and Lutge (2000) stated that inhibitory effect of distillery wastewater on germination, seedling growth, and phytotoxic effects may be due to its high salt concentration.

Phytotoxicity index

Phytotoxicity bioassays depend on measuring seed germination and root elongation (Mekki et al. 2007). Phytotoxic effect in chickpea was lowest with ETP50 ($PI = -0.05$) followed by UASBR50 ($PI = -0.02$). Highest phytotoxic effect was observed for RO25 in chickpea ($PI = 0.6$). In maize ($PI = -0.05$) and pigeon ($PI = -0.08$), lowest phytotoxicity was exhibited by UASBR50. In maize, RO10 and RO25 showed phytotoxic effect with PI value of 0.3. In pigeon pea, highest phytotoxicity effect was exhibited by RO25 treatment ($PI = 0.43$). This clearly shows that brewery wastewater from different treatment technologies at higher concentrations was phytotoxic to all three crops (Fig. 4). Brewery wastewater at lower concentration, i.e., UASBR50 and ETP50 effluent showed less phytotoxicity effect on root elongation, thus indicating significance of dilution of wastewater for reuse.

Fig. 4 Effect of brewery wastewater on phytotoxicity index of root tested crops. Means with the same letter are not significantly different from one another at 10% level of probability within different treatments

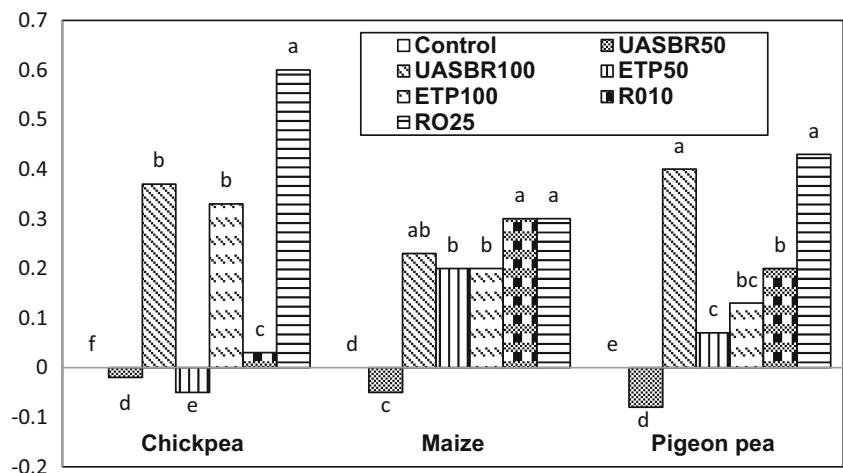
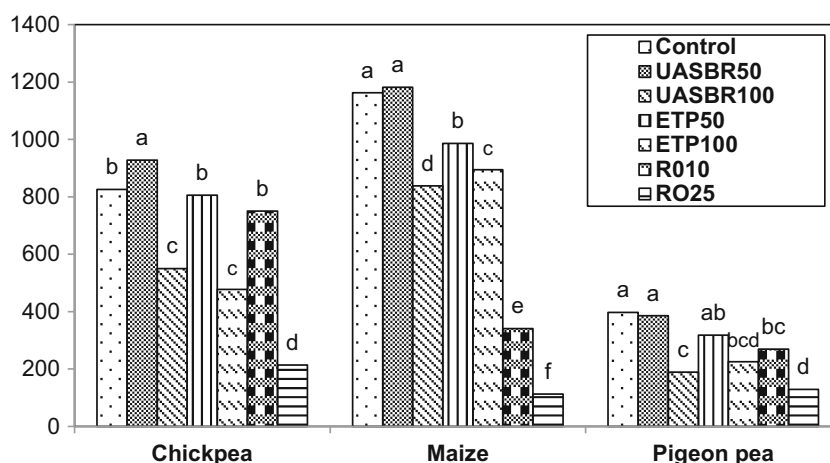


Fig. 5 Effect of brewery wastewater on vigor index of chickpea, maize, and pigeon pea. Means with the same letter are not significantly different from one another at 10% level of probability within different treatments



Similar results have been inferred by Rusan et al. (2015) using olive mill wastewater on barley seeds.

Seed vigor index

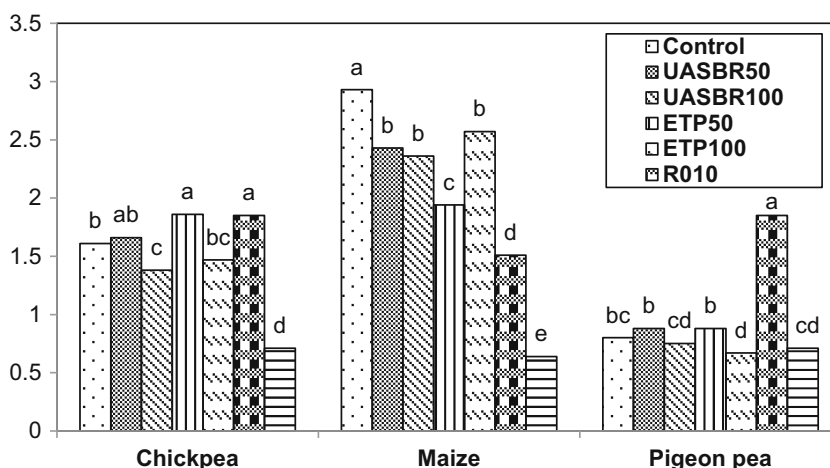
Highest SVI values of 928 and 1182 for chickpea and maize respectively were recorded with UASBR50. But in pigeon pea, highest SVI of 397 was observed with control followed by 386 for UASBR50. Lowest SVI values of 214, 113, and 129 in chickpea, maize, and pigeon pea respectively were recorded with RO25. SVI values also decreased with increased effluent concentrations. Sunflower and rice seedlings SVI values were affected by increased concentrations of synthetic oilfield-produced water (da Costa Marques et al. 2015) and municipal wastewater (Gassama et al. 2015) respectively. Diluted effluent, i.e., UASBR50, showed promoting effect than undiluted effluent on all three crops (Fig. 5). This promoting effect of UASBR50 on SVI might be due to nutrients present in it, as diluted concentrations of wastewater promotes seed quality parameters because of its lower toxicity levels and better uptake of nutrients by seedlings (Gassama et al. 2015).

Biomass

Maximum dry weight of 1.86 and 1.85 g in chickpea was recorded with ETP50 and RO10. In maize, 2.93 g of highest dry weight was recorded with control. But in pigeon pea, 1.85 g of maximum dry weight was observed with RO10. Among all treatments in chickpea and maize, minimum dry weight of 0.71 and 0.64 g respectively was recorded with RO25. But in pigeon pea, ETP100 showed minimum dry weight of 0.67 than 0.71 g of dry weight with RO25. Higher concentration of brewery effluent led to decrease in seedling dry weight (Fig. 6). Similar effects of higher concentrations of wastewater on biomass were reported by Anupama (2011) in paddy seedlings and by Narain et al. (2012a) in chickpea by using distillery effluent; by Naeem et al. (2015) on brassica seedlings using different shampoos and by Abu-Dieyeh et al. (2017) on weeds using bakery yeast wastewater.

Seed germination plays significant role in history of plant life as it affects seedling emergence, overall plant development, and plant survival. It also aids in controlling population dynamics (Wang R 2005). Seed germination bioassays along

Fig. 6 Effect of brewery wastewater on dry weight (gm) of chickpea, maize, and pigeon pea. Means with the same letter are not significantly different from one another at 10% level of probability within different treatments



with root and shoot elongation forms rapid and widely acceptable acute phytotoxicity test as it have several advantages like sensitivity, simplicity, low cost, and suitability check at initial stages itself for unstable chemicals or water samples (Zhang et al. 2015). Not only quantity but also quality of water also plays vital role for seed germination. Nowadays, due to scarcity of water, various viable options for reuse of wastewater are being explored irrespective of their quality. If water source containing higher salt concentrations are chosen, then that may lead to detrimental effect on plant growth in long run. Because in some species, seeds embryo is protected to salinity by seed coat, but after seed germination when it protrudes, then it might be injured by salts of solution/wastewater, thus leading to decreased growth (Lafragueta et al. 2014). Hence, disposal of wastewater should be done after proper treatment to remove such high salts and any other possible contaminants. Even after treatment, dilution of wastewater for irrigational purposes is regarded as best environment-friendly solution to prevent any adverse effects of wastewater on soil properties and plant growth. Germination experiments conducted by various authors led to the similar conclusion that proper dilution of wastewater is important step as their impact on germination depends on its concentration (Ramana et al. 2002; Anupama 2011; Asfi et al. 2012; Narain et al. 2012a, b) and selection of suitable crop species which is capable of tolerating wastewater should be selected.

Conclusion

From this study, it can be concluded that concentration of treated brewery effluent collected from different phases of treatment not showed any inhibitory effect on seed germination of all three tested crops, except RO25. Even though undiluted effluent of UASBR and ETP effluent showed positive effect on germination, seedling growth of three crops was promoted to the maximum by UASBR50 and ETP50. Hence, it can be recommended that dilution of brewery effluent can be recommended before using it for irrigational purpose.

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