

# Comparison of refined and non-refined wastewater effect on wheat seed germination and growth under drought

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## ABSTRACT

Wastewater has attracted special attention as a possible source of irrigation. The present study aimed to compare the effect of refined and non-refined wastewater on wheat seed germination and growth under induced drought conditions in laboratory and pot experiments. The laboratory experiment included the iso-osmotic potentials of  $-0.275$ ,  $-0.4$ , and  $-0.47$  MPa of polyethylene glycol (PEG, as a drought factor) and wastewater. In addition, the pot experiment included a wastewater factor (i.e., tap water, 100% refined wastewater, 50% refined wastewater + 50% non-refined wastewater, and 100% non-refined wastewater) and a drought factor (i.e., an irrigation interval of two and three days as normal and drought conditions, respectively). The results demonstrated that the drought related to PEG did not reduce seed germination while wastewater decreased seed germination. Further, an osmotic potential of  $-0.47$  MPa resulted in the highest and lowest radicle length in both wastewater and PEG, respectively. The results also revealed that caulicle length and seed vigour were decreased by PEG as the osmotic potential increased while no significant difference was observed between wastewater treatments and distilled water (control). Based on the results, an irrigation interval of 3 days with 100% non-refined wastewater produced the highest chlorophyll content and 100% refined and 100% non-refined wastewater produced a larger leaf area compared to the control. Furthermore, drought with wastewater application increased specific leaf weight whereas it reduced the total biomass compared to control (i.e., tap water with an irrigation interval of 2 days), except for 100% non-refined wastewater. Therefore, wastewater application compensates for the adverse effect of drought due to nutrient addition.

Keywords: chlorophyll, drought, refined wastewater, seed vigour

## INTRODUCTION

Drought is considered to be one of the most important factors which limits crop production across the globe. Agricultural production should be increased to meet global population demands (Tilman et al., 2011). Irrigation is regarded as one of the methods which effectively increases crop production while water use efficiency typically remains stable under different water amounts (Curt et al., 1995). According to Mustafa Tahir et al. (2014), shortening the irrigation interval at the critical periods of growth can lead to an increase in plant height and forage yield for oats.

Chlorophyll concentration is a key factor in photosynthesis rate (Ghosh et al., 2004), which increases under drought since leaf area reduces and the leaf becomes thicker, leading to an increase in chlorophyll concentration (Barraclough and Kate, 2001).

Wastewater (e.g., domestic and industrial types) can be used for crop irrigation. In addition, it has been found to increase plant height and biomass in wheat (Pandey and Singh, 2015). According to Ashraf and Ali (2007), seed germination is one of the sensitive stages in plant growth and, as an index of plant sensitivity to contamination, has attracted the attention of different studies. Wastewater contains salts and heavy metals. Li et al. (2005) reported that an increase in heavy metal concentration caused a decline in seed germination percentage. The highest wheat seed germination was recorded at 25% effluent concentration when compared to a variety of other concentrations (e.g., 0%, 25%, 50%, 75%, and 100%) of effluents from a textile and sugar factory (Nandal et al.,

2017). Seedling root and shoot growth decreased relative to a control when applying effluents from a pharmaceutical and battery industry at various irrigation intervals (Raju et al., 2015).

However, various studies have reported different adverse effects of salinity and drought. Both salinity and drought reduce coleoptile and root length, as well as the fresh and dry weight of the root and coleoptiles in wheat (Jovicic et al., 2018). The drought and salinity in these studies resulted from polyethylene glycol and NaCl, respectively, and had no significant effect on seed germination percentage, germination rate, or seedling shoot and root weight in wheat, compared to the control (Mohammadi and Dargahi, 2015). Farmers may use wastewater for irrigation, in refined or non-refined form, from different sources including a domestic source. Further, physical and chemical processes may be utilized for treating the wastewater (Mlakar et al., 2017). The application of non-refined wastewater for irrigation can inhibit plant growth through cell division, due to sticky and lagging chromosomes (Sik et al., 2009), and irrigating plants with non-refined wastewater may cause disease if directly applied by a person. Conversely, several other studies have indicated that non-refined wastewater can promote plant growth (Moradi et al., 2016; Khaleel et al., 2013).

It is not clear whether refined wastewater can have the same promoting effect as non-refined wastewater. Furthermore, wastewater refinement is a costly process and its efficiency needs evaluation. Therefore, the current study sought to compare physical, chemical, and biological traits of refined and non-refined wastewater and their effects on seed germination and early plant growth in wheat under drought.

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## MATERIALS AND METHODS

### Laboratory experiment

A laboratory experiment was conducted at Physiology Laboratory, the College of Agricultural Science and Engineering, Razi University, during 2014. Based on the aim of the study, the treatments encompassed the iso-osmotic potentials of  $-0.275$ ,  $-0.4$ , and  $-0.47$  MPa (equal to 100% refined wastewater, 50% refined wastewater + 50% non-refined wastewater, and 100% non-refined wastewater, respectively), of polyethylene glycol (PEG, as a drought factor) and refined or non-refined wastewater. Table 1 demonstrates some of the parameters of refined and non-refined wastewater. PEG solutions were prepared according to Michel and Kaufmann's formula (1973):

$$Y_s = -(1.18 \times 10^{-2})C - (1.18 \times 10^{-4})C^2 + (2.67 \times 10^{-4})CT + (8.39 \times 10^{-7})C^2T$$

where:  $Y_s$ ,  $C$ , and  $T$  demonstrate the osmotic potential, the concentration of PEG in  $\text{g}\cdot\text{kg}^{-1}$   $\text{H}_2\text{O}$ , and the temperature in degrees Celsius, respectively. The osmotic potentials of wastewater were measured using an osmometer and distilled water was utilized as a control. Therefore, the experiment comprised 7 treatments (i.e., one control and  $-0.275$ ,  $-0.4$ , and  $-0.47$  MPa of polyethylene glycol and wastewater). The study was conducted as a completely randomized design with 3 replications. Ghareso is a river in Kermanshah and the wastewaters from industrial and domestic sources are discharged into this river. Additionally, a wastewater treatment plant exists in Kermanshah to purify part of the effluents. In general, the purification process occurs in 3 steps: physical purification, which encompasses the construction of overflow, screens, the initial sedimentation ponds, the secondary sedimentation ponds, and the pump house of the sludge; chemical purification, which involves activated sludge and chlorination pools; and, finally, biological treatment, which involves biological reactors (Iran's Environmental Health, 2019). Wastewater samples were collected from Kermanshah wastewater treatment plant with an output of  $60\,000\text{ m}^3\cdot\text{day}^{-1}$  (Ghamarnia et al., 2014), which serves 400 000 persons.

To assess the effect of wastewater and drought on seed germination, the seeds of wheat (*Triticum aestivum* cv. Sirwan) were placed on filter paper in Petri dishes and then 6 mL of the prepared solution was added to each Petri dish. Next, these dishes were kept in a germinator for a week, after which several parameters were measured: seed germination percentage, caulicle length, radicle length, radicle to caulicle ratio, and seed vigour. Seed vigour was calculated by Heidari's (2013) equation.

### Pot experiment

An outdoor pot experiment was conducted at the College of Agricultural Science and Engineering, Razi University, in 2014. The experiment was conducted as a factorial arrangement based on a randomized complete block design with 3 replicates. One factor was wastewater (i.e., tap water, 100% refined wastewater, 50% refined wastewater + 50% non-refined wastewater, and 100% non-refined wastewater). The other factor included irrigation intervals (2 and 3 days) which were determined by a pre-experiment. In the pre-experiment, the irrigation intervals of 2 and 3 days were determined as well-watered and drought treatments, respectively, based on soil factors and plant symptoms. At each irrigation event, the soil surface was gradually watered to ensure the soil was totally wet. Then, watering was stopped when the pot soil started to drain and the seeds of the wheat (*Triticum*

*aestivum* cv. Sirwan) were sown in pots (7 cm in diameter and 7.5 cm in height) filled by field soil. The experiment lasted 21 days, after which related parameters were estimated: leaf chlorophyll content, plant height, leaf area, stem fresh and dry weight, leaf fresh and dry weight, leaf to stem ratio, total biomass, and specific leaf weight. Specific leaf weight was calculated by dividing leaf dry weight by leaf area. A SPAD (soil plant analytical development) device was used to determine the index of leaf chlorophyll (Bail et al., 2005). Finally, leaf and stem samples were dried in an oven at  $70^\circ\text{C}$  for 24 h in order to calculate their dry weight.

### Data analysis

Data were analysed by SAS software and the means were compared by applying Duncan's multiple range test at a probability level of 5%.

## RESULTS

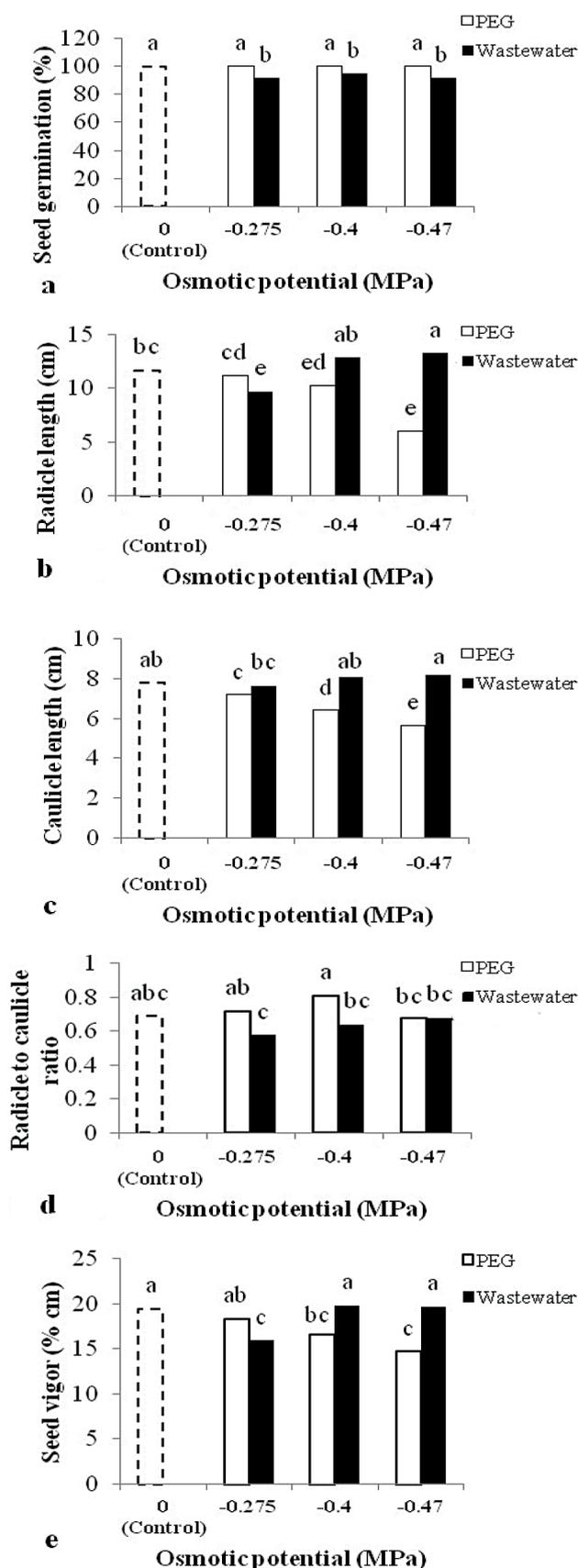
### Water quality

The effluent quality was improved by the wastewater treatment plant (Table 1). For example, heavy metals, as well as some essential nutrients for plant growth, such as nitrogen and

**Table 1.** Non-refined and refined wastewater parameters (Dindarlou and Dastourani, 2018; Ghamarnia et al., 2014)

Parameter	Non-refined wastewater	Refined wastewater
$\text{NO}_3^-$ ( $\text{mg}\cdot\text{L}^{-1}$ )	70	30
$\text{PO}_4^{3-}$ ( $\text{mg}\cdot\text{L}^{-1}$ )	15.6	4.3
K ( $\text{mg}\cdot\text{L}^{-1}$ )	8.6	8.7
Na ( $\text{mg}\cdot\text{L}^{-1}$ )	45	23
Ca ( $\text{mg}\cdot\text{L}^{-1}$ )	277.9	144.2
Mg ( $\text{mg}\cdot\text{L}^{-1}$ )	98.4	48.6
K ( $\text{mg}\cdot\text{L}^{-1}$ )	8.6	4.3
Al ( $\text{mg}\cdot\text{L}^{-1}$ )	6.3	5.3
Cl ( $\text{mg}\cdot\text{L}^{-1}$ )	63.5	39.28
B ( $\text{mg}\cdot\text{L}^{-1}$ )	0.5	0.04
Hg ( $\text{mg}\cdot\text{L}^{-1}$ )	0.08	0.009
Fe ( $\text{mg}\cdot\text{L}^{-1}$ )	10.5	22.11
Cu ( $\text{mg}\cdot\text{L}^{-1}$ )	0.05	0.01
Zn ( $\text{mg}\cdot\text{L}^{-1}$ )	1.3	0.1
Cd ( $\text{mg}\cdot\text{L}^{-1}$ )	0.09	0.05
Ni ( $\text{mg}\cdot\text{L}^{-1}$ )	0.06	0.03
Cr ( $\text{mg}\cdot\text{L}^{-1}$ )	0.07	0.02
Pb ( $\text{mg}\cdot\text{L}^{-1}$ )	0.09	0.01
Mn ( $\text{mg}\cdot\text{L}^{-1}$ )	3.2	4.3
Co ( $\text{mg}\cdot\text{L}^{-1}$ )	0.05	0.04
As ( $\text{mg}\cdot\text{L}^{-1}$ )	0.07	0.02
Se ( $\text{mg}\cdot\text{L}^{-1}$ )	0.1	0.04
Total suspended solids ( $\text{mg}\cdot\text{L}^{-1}$ )	83.8	20.4
Total dissolved solids ( $\text{mg}\cdot\text{L}^{-1}$ )	801.7	619.4
Total organic carbon ( $\text{mg}\cdot\text{L}^{-1}$ )	62.4	19.3
Total hardness ( $\text{mg}\cdot\text{L}^{-1}$ )	1 104.75	563.9
Sodium adsorption ratio	3.28	2.67
Turbidity (NTU)	69.6	39.4
EC ( $\text{ds}\cdot\text{m}^{-1}$ )	0.429	0.76
pH	6.33	7.9
Total coliform (MPN 100 $\text{mL}^{-1}$ )	1 500	95

NTU: nephelometric turbidity units; EC: electrical conductivity



**Figure 1.** The effect of the iso-osmotic potentials of PEG and wastewater on seed germination traits in wheat. Means with the same letter represent no significant differences ( $P < 0.05$ ).

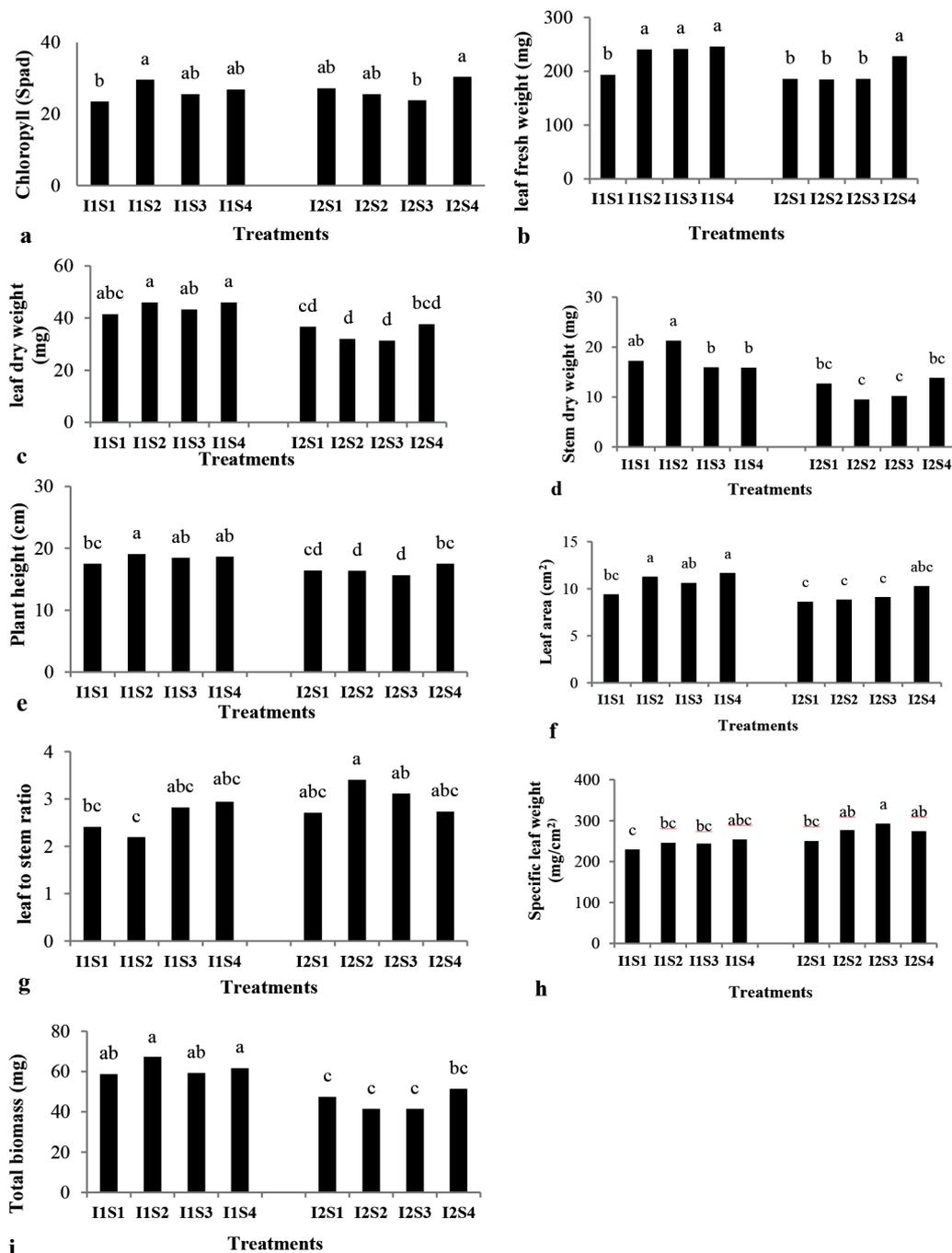
phosphorus, were reduced by wastewater treatment. Fe increase could be attributed to  $\text{FeCl}_3$  for coagulation. Mn increase could be due to the lack of subsurface oxygen. pH increase could be attributed to liming.

### Laboratory experiment

Figure 1 illustrates the influence of polyethylene glycol (PEG) iso-osmotic potentials and wastewater on the seed germination traits of wheat. Drought related to PEG fails to reduce seed germination while wastewater decreases it (Fig. 1a). Further, germination reduction by wastewater is initiated by the lowest osmotic potential. The osmotic potential of  $-0.47$  MPa for the wastewater and PEG resulted in the highest and lowest radicle length, respectively (Fig. 1b). An increase in osmotic potential leads to a decrease in the caulicle length with PEG, whereas no significant difference is observed between wastewater treatments and distilled water as control (Fig. 1c). With regard to the radicle to caulicle ratio, there is also no significant difference between wastewater treatments and distilled water (Fig. 1d). Furthermore, seed vigour decreases with PEG through increasing the osmotic potential, while wastewater treatments and distilled water demonstrate no significant difference in terms of seed vigour (Fig. 1e).

### Pot experiment

The effect of irrigation interval and wastewater on early growth traits in wheat is displayed in Fig. 2. The irrigation interval of 3 days with 100% non-refined wastewater produces the highest chlorophyll content. More precisely, drought reduces leaf area while increasing leaf thickness (specific leaf weight), leading to an increase in chlorophyll concentration which is confirmed by the results of the current experiment (Fig. 2a). Under the irrigation interval of 2 days, leaf fresh weight increases for all wastewater treatments whereas under the irrigation interval of 3 days, only 100% non-refined wastewater results in an increase in leaf fresh weight (Fig. 2b). Drought reduces leaf dry weight (Fig. 2c). Additionally, 100% refined wastewater produces the highest stem dry weight. A remarkable reduction of some nutrients while improving some physiochemical properties of wastewater (e.g., electrical conductivity) in 100% refined wastewater results in increasing the stem dry weight (Fig. 2d). In addition, 100% refined wastewater with an irrigation interval of 2 days reveals higher plant height compared to the control, namely, tap water with an irrigation interval of 2 days. By increasing the irrigation interval, the height of the plant irrigated by 100% non-refined wastewater shows no reduction (Fig. 2e), which is likely due to the positive effect of wastewater on plant growth. Further, 100% refined and 100% non-refined wastewater produce a higher leaf area compared to the control (Fig. 2f). The lowest and highest leaf to stem ratio, among the treatments, is observed for 100% refined wastewater with an irrigation interval of 2 and 3 days, respectively (Fig. 2g). On the other hand, drought increases leaf to stem ratio under 100% refined wastewater. This suggests that stem elongation initiation is postponed by drought. Additionally, drought with wastewater application leads to an increase in specific leaf weight compared to the control (Fig. 2h), and finally, drought reduces total biomass compared to the control, except in the case of 100% wastewater (Fig. 2i).



**Figure 2.** The effect of irrigation interval and wastewater on early growth traits in wheat. Note: S1, S2, S3, and S4 represent tap water, 100% refined wastewater, 50% refined wastewater + 50% non-refined wastewater, and 100% non-refined wastewater, respectively. In addition, I1 and I2 are the irrigation intervals of 2 and 3 days. Means with the same letter indicate no significant differences ( $P < 0.05$ ).

## DISCUSSION

### Laboratory experiment

The reduction in seed germination by wastewater can be attributed to salinity and heavy metal stresses, which is confirmed by the data in Table 1. Further, higher electrical conductivity and some inhibiting elements, including Na, in the wastewater are the main reasons for such a reduction in seed germination. Apparently, salinity stress had a negative effect on germination while some nutrients promoted radicle

and caulicle length after germination in the present study. However, these nutrients demonstrated their effect only at high concentration. The adverse effect of osmotic potential was compensated for by the positive effect of wastewater due to nutrients (Li et al., 2005). Raju et al. (2015) also reported a decline in wheat seed germination due to effluent from a pharmaceutical and battery industry, which is in line with the results of the current study. Furthermore, some other studies found that seed priming by sodium compounds such as sodium silicate (Hameed et al., 2013) and sodium nitroprusside (Ali et al., 2017) enhance seedling root and shoot growth in wheat

due to a reduction in the oxidative stress of salinity. This corroborates the results of the present study. Contrary to the results of the current study, Sayar et al. (2010) concluded that drought stress resulting from polyethylene glycol (PEG) had a higher adverse effect on germination percentage in wheat compared to NaCl-related salinity. Additionally, PEG-induced drought stress had an even more negative effect on radicle to caulicle ratio compared to wastewater (Fig. 1d). Essential nutrients available in wastewater reduced the negative effects of drought. Saidi et al. (2010) reported that root weight increases in wheat seedlings under increasing drought. Seed treated with wastewater represented higher seed vigour under the osmotic potential of  $-0.4$  and  $-0.47$  MPa, compared to the seed treated with PEG (Fig. 1e). The results of another study indicated that NaCl had a more adverse effect on germination compared to drought (Al-taisan, 2010) while other studies have reported that drought had a more adverse effect on germination compared to NaCl (e.g., Okcu et al., 2005; Rahimi et al., 2006).

### Pot experiment

Heidari et al. (2011) found an increase in the chlorophyll content of water-stressed plants while Abdalla and El-khoshiban (2007) reported that drought reduced the chlorophyll content. This contradiction can be attributed to stress severity. In other words, chlorophyll is destroyed under severe drought which leads to a decrease in its content. In this respect, the effect of wastewater should be considered as well. Wastewater contains essential nutrients, including nitrogen, for plant growth and chlorophyll formation, and chlorophyll content, as one of source strength components, is affected by wastewater (Ghosh et al., 2004). In addition, 100% non-refined wastewater contains more nutrients than tap water and refined wastewater (Table 1). Leaf fresh weight mainly relies on leaf moisture and its trend indicates that wastewater salts may be absorbed by plant tissues. Further, salts accumulate water, thus the leaf becomes succulent (Sen and Rajpurohit, 2012) and drought reduces leaf dry weight. In the present study, the comparison of leaf dry and fresh weight revealed that wastewater increased leaf moisture (Fig. 2). According to Alyemny (1998), a reduction in leaf biomass helps the plant to tolerate water deficit, and plant height reduction as a result of drought is considered as an adaptation response of plants in order to reduce transpiration (Karam et al., 2003). Furthermore, drought decreases cell size and internode length (Ludlow et al., 1990). However, based on the results of the current study, drought did not reduce leaf area for the 100% non-refined wastewater treatment (Fig. 2). A larger amount of nutrients such as nitrogen and phosphorus in 100% non-refined wastewater plays a role in chlorophyll production and increasing leaf area, compared to refined wastewater. Additionally, drought diminishes cell elongation and division, and thus reduces leaf area. Other studies, including Karam et al. (2003), have reported leaf area reduction due to drought as well. In addition, the results of the present study confirm that drought reduces the leaf area and increases the leaf thickness, leading to an increase in specific leaf weight (Fig. 2). The results further indicate that wastewater can promote plant growth by increasing the leaf thickness in order to capture more radiant energy. Water deficit leads to various morpho-physiological changes in the plants. For example, leaf growth is inhibited first, before photosynthesis and respiration, when there is a reduction in water potential, and soil water deficit reduces xylem sap movement toward the leaves (Liptay et al., 1998).

## CONCLUSIONS

In general, an osmotic potential of  $-0.47$  MPa of wastewater, resulted in the lowest seed germination percentage. Further, wastewater at low water potential failed to reduce seed germination traits such as seed vigour, while using polyethylene glycol to create a low osmotic potential decreased these traits. It is likely that the positive physiochemical properties of wastewater, including nitrogen content, acted to promote plant growth. Furthermore, plants irrigated with 100% refined wastewater under the well-watered condition produced the highest total biomass, since 100% refined wastewater contained growth-promoting ingredients such as nitrogen and phosphorus. Based on the results, drought reduced different growth parameters such as leaf and stem dry weight whereas wastewater application compensated for the negative effect of drought. Finally, drought decreased total biomass compared to control, except in the case of the 100% wastewater treatments. Therefore, irrigation of wheat with wastewater is recommended after germination instead of at the germination stage, and irrigation with refined wastewater can promote plant growth.

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