

Effect of recycled water applied by surface and subsurface irrigation on the growth, photosynthetic indices and nutrient content of young olive trees in central Iran

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ABSTRACT

Water shortage has encouraged the quest for alternative sources of water for food production and agricultural development. Recycled water (RW) is one of the most available water resources with great potential for use in farm irrigation. This experiment was carried out to investigate the use of RW as the irrigation source and its application method, subsurface leaky irrigation (SLI) system or surface irrigation, in an orchard with young olive trees in central Iran. The results revealed that the SLI system was able to enhance tree growth, leaf area, maximum fluorescence (Fv/Fm) and photosynthesis rate by 68%, 26%, 4%, and 42%, respectively. In addition, trees irrigated with the SLI system using RW exhibited increased N and Mg uptakes by 138% and 8%, respectively. Plants irrigated with RW showed a growth improvement (42%), leaf area (26%), and photosynthesis rate (23.4%) compared with those irrigated with clean water. Furthermore, Mg, Na, K, P, and N content increased by 12%, 59%, 30%, 7%, and 92%, respectively, in leaf tissue when RW was applied. The results indicated that RW could be employed as a reliable irrigation source especially when it was delivered with the SLI system.

Key words | chlorophyll fluorescence, nutrition element, recycled water

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INTRODUCTION

Water shortage in (semi-)arid regions and its declining availability to a critical level on a global scale dictates the reliance of sustainable and increasing agricultural production on alternative water resources for irrigation (Nirit *et al.* 2006). Recycled water (RW) is an important alternative source of water for irrigation, which will increase with the growing population and the increasing demand for freshwater (Hassanli *et al.* 2008). Reusing RW in irrigation has been widely practiced in many countries, especially in (semi-)arid regions. Increasing water reuse as a result of the growing urbanization in Iran, which is located in a dry region, can be considered as the optimal use of RW to

overcome the present water crisis. Moreover, urban wastewater can be used not only as a source of irrigation water but also as a source of nutrients for plants, thanks to its large supply of nutrients and organic matter (Meli *et al.* 2002; Ratton *et al.* 2005; Singh & Bhati 2005; Aghabarati *et al.* 2008; Tak *et al.* 2013). Because water shortage is a limiting factor for increased agricultural production and green-space development, water reuse may be a useful remedy.

When water supplies are restricted, improving irrigation methods to meet water demand becomes important. Malash *et al.* (2005) reported that drip irrigation has a higher efficiency in irrigating different tomato varieties than the traditional methods such as flooding irrigation. Al-Omran *et al.* (2010) observed that drip irrigation using saline water would be more useful with more frequent irrigations in order to reduce salt concentration in the root zone.

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doi: 10.2166/wrd.2016.019

Although drip irrigation is considered to be suitable for RW reuse, its efficiency is mainly limited by emitter clogging (Capra & Scicolone 2004). This limitation justifies the application of other irrigation systems such as subsurface leakage systems.

Olive (*Olea europaea* L.) is an evergreen tree grown for both oil production for human consumption and for processing as table olives (Fernandez-Escobar *et al.* 2006) with a high degree of drought tolerance. This species can adapt itself to water scarcity through modification of its leaf area as well as its morphological, anatomical, and physiological characteristics (Bacelar *et al.* 2006). Olive is one of the most important crops among the horticultural species in Iran. Since almost all the orchards in Iran, including olive ones, are irrigated with fresh water, no information is available about the effects of RW on the growth, production, and quality of olives.

The objectives of this study were: (1) to assess the nutritional value of RW and its usage possibility in olive orchard irrigation; and (2) to evaluate the effects of subsurface leakage irrigation system for RW application and its effect on young olive trees establishment and growth.

MATERIALS AND METHODS

This experiment was conducted in the Department of Horticulture, Isfahan University of Technology, Isfahan (51 °W, 31 °N; Altitude 1600 m), Iran, during 2010–2012, using an orchard with a sandy-clay soil (pH = 7.3, EC = 2.3 ds/m). The climate of the area is arid with cold winters. Average annual rainfall and average annual maximum temperature are 122.8 mm and 23.4 °C, respectively. The experiment was conducted using a split-plot experimental design with two factors and four replicates. The treatments included: irrigation systems – subsurface leaky irrigation (SLI), or surface irrigation (SI); and water quality – RW, or clean water (CW). Young olive trees (*Olea europaea* L., cv Roghani) (1 year old) were planted at a spacing of 4.0 m by 4.0 m. The trees were irrigated for two consecutive years based on crop evapotranspiration rate (ET_c, mm) of about 173.4 L/mo per tree (data not shown). The characteristics of the urban RW used in the experiment are shown in Table 1.

Table 1 | Chemical characteristics of the RW and CW used for the irrigation of the young olive trees in this study

| Constituent (unit) | RW | CW |
|---|--------|------|
| K (mg l ⁻¹) | 136.50 | – |
| Na (mg l ⁻¹) | 118.80 | 1.20 |
| P (mg l ⁻¹) | 1.80 | – |
| NO ₃ ⁻ -N (mg l ⁻¹) | 1.68 | – |
| SO ₄ ²⁻ (mg l ⁻¹) | 108.96 | – |
| CaCO ₃ (mg l ⁻¹) | 149.45 | – |
| pH | 8.62 | 7.40 |
| EC (dS m ⁻¹) | 1.00 | 0.59 |

Water was distributed in the soil through a 10.16 cm diameter drainage pipe in the SLI system. The tubes were wrapped in a soft polyester yarn to prevent clogging with soil. The drainage pipes (Ahwaz Pipe Mills Co., Ahwaz, Iran) were installed at a depth of 30–40 cm below the soil surface, and 40 cm away from each row of trees. Tube length in the SLI irrigation (1 m per tree) was designed based on the tree canopy using a linear arrangement. The vegetative growth was determined at the end of each month by measuring the difference in tree height at the end of each month.

The leaf area was determined using a leaf area meter DT-scan (Delta-Scan Version 2.03, Delta-T Devices Ltd, Burwell, Cambridge, UK). Plant chlorophyll content was measured with a spectrophotometer according to the method developed by Lichtenthaler (1987) and the free proline content was determined according to the method described by Bates *et al.* (1973).

Net photosynthesis rate (P_n), internal CO₂, partial pressure (C_i), and stomatal conductance (g_s) were measured in the morning (09:30–11:30) using a portable gas exchange system (Li-6400, LICOR, Lincoln, NE, USA) on the fully expanded leaves situated at the mid-canopy of the plants. The fluorescence measurements (F_v/F_m) were performed using a modulated fluorometer (RS232, Hansatech, Instruments Ltd, UK).

Leaf nutrient content measurements were carried out using the samples collected in July as the best time for diagnosing tree nutritional status (Fernandez-Escobar *et al.* 2006). After drying, ashing, and digestion in chloridric acid, the mineral content of leaves was determined. Na and K

were determined using a flame photometer (PFP7, Jenway, UK). Zn and Mg concentrations were determined by atomic absorption photometry (Perkin Elmer AA3030, Norwalk, CT). The Kjeldahl procedure was used to determine total N (Bremner 1996). P was estimated by colorimetric determinations using a spectrophotometer (UV-160, A, Shimadzu, Japan). The collected data were subjected to the analysis of variance using the SAS (9.1, SAS institute, 2004) statistical software. Statistical assessments of differences between mean values were performed by the least significant difference (LSD) test at $P = 0.05$.

RESULTS

Table 2 presents the effects of water quality on vegetative measurements and photosynthetic indices of olive trees. Clearly, no significant differences were observed between the 2 years of study for g_s and the number of shoots; however, P_n , height and chlorophyll content showed higher values with RW application. There was no significant

difference between the tree height and the leaf area between the RW and the CW in the first year, but they increased by 42% and 29%, respectively, with RW application in the second year. Chlorophyll content in plants receiving RW was higher by 28% and 12% in the first and second year, respectively. Photosynthetic rate was significantly higher ($23.4 \mu\text{mol m}^{-2} \text{s}^{-1}$) in plants receiving RW compared to those receiving CW (Table 2).

It can be observed in Table 3 that, except for the number of shoots, neither the P_n indices nor the growth parameter exhibited any significant differences in the first year between the two treatments. One year after the initiation of the irrigation treatments, the plants irrigated with the SLI system exhibited remarkable increases in their photosynthetic indices and growth rates (Table 3). Statistically significant differences were also observed in the photosynthetic and vegetative indices between plants irrigated with the subsurface irrigation system and those irrigated with the surface system. The results indicated that, compared with the SI system, the SLI system increased P_n , leaf area, height, and number of shoots by 42%, 26%, 68% and 19%, respectively.

Table 2 | Vegetative and photosynthetic indices of young olive trees as influenced by water quality in the 2 years of study

| Treatment | | Height (cm) | Leaf area (mm ²) | Chlorophyll ab. (mg.g ⁻¹) | Number of shoots | P_n ($\mu\text{mol.m}^{-2}\text{s}^{-1}$) | E (Mmol.m ⁻² s ⁻¹) | g_s ($\mu\text{mol.m}^{-2}\text{s}^{-1}$) | Sugar (mg.g ⁻¹) |
|-----------|----|--------------------|------------------------------|---------------------------------------|--------------------|---|---|---|-----------------------------|
| 2011 | CW | 12.2 ^a | 351.35 ^a | 2.1 ^b | 13.05 ^a | 18.3 ^b | 2.95 ^b | 0.21 ^a | 26.6 ^a |
| | RW | 10.56 ^a | 386.8 ^a | 2.71 ^a | 8.6 ^a | 23.6 ^a | 4.0 ^a | 0.26 ^a | 19.8 ^b |
| LSD | | 4.45 | 56.18 | 0.5 | 4.98 | 4.28 | 0.94 | 0.09 | 4.74 |
| 2012 | CW | 14.7 ^b | 322.35 ^b | 1.30 ^b | 22.6 ^a | 17.57 ^b | 4.11 ^a | 0.23 ^a | 16.45 ^a |
| | RW | 20.9 ^a | 418.36 ^a | 1.46 ^a | 22.8 ^a | 23.4 ^a | 4.72 ^a | 0.28 ^a | 17.12 ^a |
| LSD | | 6.01 | 95.1 | 0.11 | 2.8 | 5.6 | 1.55 | 0.11 | 3.2 |

Note: Values with the same letter are not significantly different at 5% probability level according to the LSD test.

Table 3 | Vegetative and photosynthetic indices of olive trees influenced by irrigation system in 2 years of study

| Treatment | | Height (cm) | Leaf area (mm ²) | Chlorophyll ab. (mg.g ⁻¹) | Number of shoots | P_n ($\mu\text{mol.m}^{-2}\text{s}^{-1}$) | E (Mmol.m ⁻² s ⁻¹) | g_s ($\mu\text{mol.m}^{-2}\text{s}^{-1}$) | Sugar (mg.g ⁻¹) |
|-----------|-----|--------------------|------------------------------|---------------------------------------|--------------------|---|---|---|-----------------------------|
| 2011 | SI | 11.75 ^a | 379.22 ^a | 2.34 ^a | 6.7 ^b | 23.21 ^a | 3.55 ^a | 0.218 ^a | 23.33 ^a |
| | SLI | 11.03 ^a | 359 ^a | 2.53 ^a | 14.9 ^a | 18.77 ^a | 3.46 ^a | 0.259 ^a | 23.15 ^a |
| LSD | | 9.9 | 50.95 | 0.92 | 6.61 | 6.42 | 1.96 | 0.099 | 10.12 |
| 2012 | SI | 13.26 ^b | 327.5 ^b | 1.36 ^b | 20.9 ^b | 16.9 ^b | 3.92 ^b | 0.21 ^b | 20.03 ^a |
| | SLI | 22.34 ^a | 413.2 ^a | 1.47 ^a | 24.93 ^a | 24 ^a | 4.92 ^a | 0.30 ^a | 13.6 ^b |
| LSD | | 2.94 | 48.19 | 0.11 | 2.16 | 4.9 | 0.61 | 0.06 | 5.85 |

Note: Values with the same letter are not significantly different at 5% probability level according to the LSD test.

Sugar content in the SI system, however, was 47% higher compared to that of the SLI system (Table 3).

Figure 1 shows the effects of irrigation and water quality treatments on the vegetative growth. Clearly, no significant differences were observed between the two treatments with respect to the tree growth in the first year, but using RW increased the vegetative growth by 26% in the SLI and by 20% in the SI treatments at the end of the second year. In addition, the SLI system using RW led to a better growth rate (54%) than did the SI system (Figure 1).

Using RW for subsurface irrigation increased Na, Mg, and N contents in the leaf tissue. As shown in Table 4, irrigation with RW, as compared with CW, enhanced N and Mg contents in leaf tissue by 138% and 8%, respectively, in the SLI treatment and by 38% and 12%, respectively, in the SI system (Table 4).

Leaf content of Zn, K, and Na showed no significant differences between the irrigation treatments; Mg, P, and N, however, increased by 9%, 12%, and 45%, respectively, in the subsurface irrigation system over the 24 months of the experiment. Proline content increased significantly in subsurface leakage irrigation throughout the experiment period. K and Zn concentrations in the leaf tissue remained unaffected by irrigation system treatments (Table 5). A tendency was observed for an increasing Fv/Fm ratio (maximum fluorescence) in plants irrigated with the SLI system compared to the SI system (Table 5).

RW significantly increased proline, Na, Mg, N, and P concentrations in the leaves of the olive trees compared with CW. Irrigation with RW for 24 months enhanced Mg, Na, K, P, and N concentrations in the leaf tissue by 12%, 59%, 30%, 7%, and 92%, respectively, as compared with CW. Moreover, trees irrigated with RW exhibited an enhanced Fv/Fm intensity by 4% (Table 6). Finally, the proline content of leaf tissue increased (23%) in plants irrigated for 24 months with RW compared to those irrigated with CW.

DISCUSSION

In this experiment, the growth improved in young olive trees receiving RW, indicating the positive effect of RW on the plant growth and photosynthetic rate. Generally speaking, RW can be a rich source of nutrients required for plant growth (Toze 2006). Our results are confirmed by those of Aghabarati et al. (2008) who reported that the application of municipal RW led to better growth in olive trees. The enhanced growth may be attributed to the availability of nutrients and the adequacy of the water available in soil for meeting plant water demand. Leaf area was higher in the SLI system with RW application. Large leaf area is of great importance for light interception and Pn in plants, leading to enhanced photosynthetic rate and growth. A

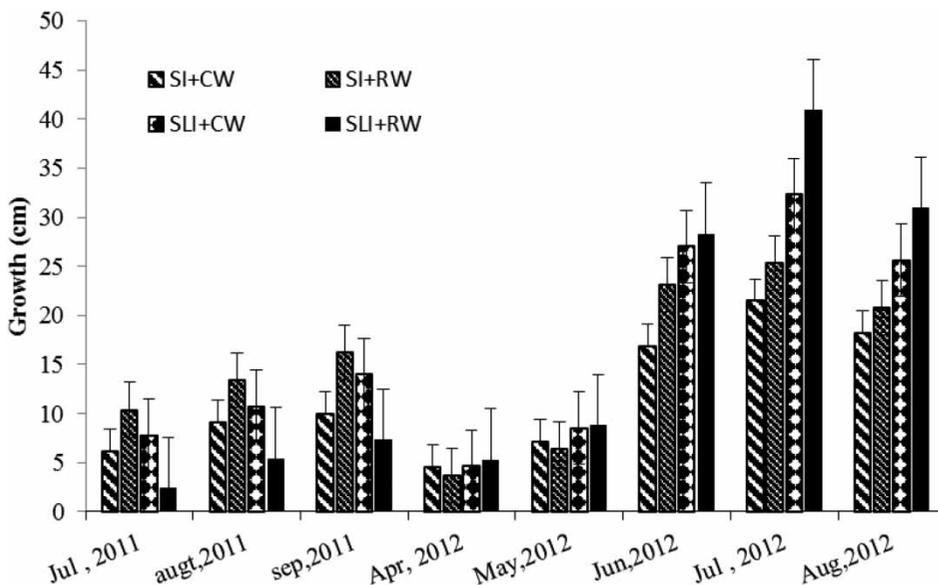


Figure 1 | Growth in young olive trees during the experimental period.

Table 4 | Nutritional content of olive trees influenced by water quality and irrigation system

| Treatment | | Proline ($\mu\text{mol g}^{-1}$) | Na (mg.g^{-1}) | Zn (mg.g^{-1}) | K (mg.g^{-1}) | P (mg.g^{-1}) | Mg (mg.g^{-1}) | N (mg.g^{-1}) |
|-----------|----|------------------------------------|---------------------------|---------------------------|--------------------------|--------------------------|---------------------------|--------------------------|
| SI | CW | 0.31 ^a | 1.07 ^c | 0.8 ^b | 2.71 ^{ab} | 2.32 ^b | 2.1 ^b | 1.01 ^b |
| | RW | 0.34 ^a | 1.49 ^b | 0.14 ^a | 3.39 ^a | 2.63 ^a | 2.37 ^{ab} | 1.43 ^b |
| SLI | CW | 0.21 ^b | 1.01 ^c | 0.1 ^b | 2.49 ^b | 2.76 ^a | 2.32 ^{ab} | 1.05 ^b |
| | RW | 0.30 ^a | 1.84 ^a | 0.14 ^a | 3.37 ^{ab} | 2.85 ^a | 2.51 ^a | 2.52 ^a |
| LSD | | 0.085 | 0.308 | 0.030 | 0.807 | 0.309 | 0.285 | 0.503 |

Note: Values with the same letter are not significantly different at 5% probability level, according to the LSD test.

Table 5 | Nutrient content in olive leaf as affected by the irrigation systems

| Treatment | Fv/Fm | Proline ($\mu\text{mol.g}^{-1}$) | Na (mg.g^{-1}) | Zn (mg.g^{-1}) | K (mg.g^{-1}) | P (mg.g^{-1}) | Mg (mg.g^{-1}) | N (mg.g^{-1}) |
|-----------|--------------------|------------------------------------|---------------------------|---------------------------|--------------------------|--------------------------|---------------------------|--------------------------|
| SI | 0.722 ^b | 0.32 ^a | 1.28 ^a | 0.115 ^a | 2.93 ^a | 2.48 ^b | 2.23 ^b | 1.22 ^b |
| SLI | 0.75 ^a | 0.25 ^b | 1.42 ^a | 0.127 ^a | 3.05 ^a | 2.80 ^a | 2.45 ^a | 1.78 ^a |
| LSD | 0.02 | 0.06 | 0.678 | 0.021 | 0.571 | 0.219 | 0.201 | 0.355 |

Note: Values with the same letter are not significantly different at 5% probability level, according to the LSD test.

Table 6 | Comparison of the accumulation of nutrient element in olive leaf in water quality treatments

| Treatment | Fv/Fm | Proline ($\mu\text{mol.g}^{-1}$) | Na (mg.g^{-1}) | Zn (mg.g^{-1}) | K (mg.g^{-1}) | P (mg.g^{-1}) | Mg (mg.g^{-1}) | N (mg.g^{-1}) |
|-----------|-------------------|------------------------------------|---------------------------|---------------------------|--------------------------|--------------------------|---------------------------|--------------------------|
| CW | 0.72 ^b | 0.26 ^b | 1.04 ^b | 0.127 ^a | 2.6 ^b | 2.54 ^b | 2.21 ^b | 1.03 ^b |
| RW | 0.75 ^a | 0.32 ^a | 1.66 ^a | 0.115 ^a | 3.38 ^a | 2.74 ^a | 2.47 ^a | 1.98 ^a |
| LSD | 0.03 | 0.05 | 0.49 | 0.015 | 0.686 | 0.094 | 0.196 | 0.599 |

Note: Values with the same letter are not significantly different at 5% probability level, according to LSD test.

decline in leaf area, however, is considered as one of the plant resistance mechanisms under water shortage (Guarnaschelli *et al.* 2003).

The application of wastewater is recommended for orchard irrigation as it is a rich source of N, P and K that contribute to enhanced leaf area and biomass production (Guo & Sims 2000).

Some researchers have suggested a direct relationship between pigment concentration, which is, in turn, directly related to photosynthetic activity, and environmental factors. Herteman *et al.* (2011) reported that the application of RW enhances chlorophyll pigment concentration in mangrove leaves. N and Mg are known to be the nutrients that are necessary for the synthesis of chlorophyll (Suntoro 2002). Availability of these nutrients can increase the chlorophyll content on which the photosynthetic rate directly

depends (Suharja & Suntoro 2009). Chlorophyll content and photosynthetic rates have been found to have a correlation. There are some reports indicating that an increase in available N increased photosynthetic rates for different mangrove trees (Li *et al.* 2008; Herteman *et al.* 2011). Wastewater application reportedly led to enhanced photosynthetic rates, stomatal conductance (gs), and vegetative growth in chickpeas because of the dual effect of wastewater, it is not only a valuable source of water but also a rich source of nutrients (Tak *et al.* 2013; Ashrafi *et al.* 2015). Enhanced concentration and uptake of these nutrients by plants through irrigation with RW were the major factors in enhanced chlorophyll content, photosynthetic rate, leaf area, and plant height.

Application of wastewater, compared with CW, significantly increased N, P, K, Mg, and Na concentrations in

olive trees. This result is in agreement with the findings of Singh & Bhati (2005) and Aghabarati *et al.* (2008) who observed higher concentrations of minerals in the leaves of *Dalbergia sissoo* and olive trees irrigated with wastewater. Increased concentrations of N, P, and K in the RW leads to the accumulation of nutrients in soil and makes them readily available to plants.

Chlorophyll fluorescence (Fv/Fm) ratio is widely used to detect stress conditions and to determine the photosynthetic performance of the plant. The Fv/Fm ratio is a useful guide for the evaluation of maximum quantum efficiency of PSII. It has been widely used to discover stress-induced perturbations in the photosynthetic device (Baker & Eva 2004). Souza *et al.* (2004) showed that a decrease in maximum quantum yield of PSII (Fv/Fm) was observed at an advanced phase of stress. Our result is in agreement with these findings.

Some of the differences observed in tree performance are directly related to differences in the irrigation efficiency of different irrigation systems. Trees irrigated by surface dripping have been observed to be significantly larger with higher yields than those irrigated by other systems when the same amount of water was applied (David *et al.* 2003). Subsurface drip systems may further improve upon the efficiencies of irrigation and fertilizer application because water and nutrients are supplied directly to the root zone (Camp 1998). Interaction effect of recycle water and SLI resulted in slower growth in the first year. Probably, this stems from the fact that trees need to develop their root systems and adapted to the SLI system to take advantage of the water and nutrients available in the SLI system and RW.

Photosynthetic indices decline is one of the initial harmful effects of water shortage (Lawlor & Cornic 2002). Lower Pn and Ci under SI compared with the SLI system in the present study could be possibly due to water shortage. Previous studies reported different Pn rate under different irrigation treatments in the rice plant (Zhou *et al.* 2003; Dass & Chandra 2013).

The SLI system used in the present study was found to have the potential to save irrigation water by creating more favorable conditions in the root zone and to improve water availability by reducing evaporation. These results are in agreement with those reported in Al-Omran *et al.* (2005), Al-Omran *et al.* (2010) and Yang *et al.* (2011).

Sakellariou-Makrantonaki *et al.* (2007) reported larger leaf area indices in plants irrigated with surface and subsurface drip irrigation methods compared with those irrigated by the other methods.

Soluble sugars accumulation increased in the leaves of young olive trees under the SI system. It is commonly observed that water stress conditions induce an accumulation of soluble sugars in leaves that may act as compatible solutes while they are sources of carbon for plant growth and maintenance (Chaves *et al.* 2002).

Based on the results of the present study, it may be concluded that trees exhibited enhanced growth rates when irrigated with RW. This is mostly due to the high nutrient concentrations in RW. Also, the subsurface leakage method outperforms the surface method in improving plant growth index and photosynthetic rates in young olive trees in central Iran. Further in-depth study is, therefore, suggested for a deeper understanding of RW used for orchard irrigation.

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First received 12 January 2016; accepted in revised form 15 March 2016. Available online 23 April 2016