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Effect of treated wastewater reuse at different soil moisture on growth and essential oil yield and percentage of sage grown in degraded soil

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ABSTRACT

A pot experiment was conducted to investigate the effects of two irrigation water sources: treated wastewater (TWW) and fresh water and two soil moisture levels (100% and 60% of available water capacity (AWC) on the essential oil yield and percentage and growth parameters of sage plants (*Salvia officinalis* L.) grown in degraded soil. Sage plants were cultivated for 90 and 180 days at planting densities of 70 and 50 plants per pot, respectively. Despite both water sources being classified as C3-S1, no significant short-term effects on soil properties were observed after 90 or 180 days. After 90 days, TWW at 100% AWC significantly increased plant height and leaf fresh and dry weight compared to other treatments. Similar trends were observed at 180 days, regardless of planting density. Additionally, SPAD significantly increased at 100% AWC, regardless of water source. Treatments significantly affected the essential oil yield and percentage of sage plants grown for 90 and 180 days. TWW at 100% AWC resulted in the highest essential oil yield, which was significantly higher than other treatments. Unexpectedly, irrigation with TWW at 60% AWC resulted in the highest oil percentage after 90 days. However, after 180 days, TWW at 100% AWC led to a significantly higher essential oil percentage compared to fresh water at 100% AWC. Under the conditions of fresh irrigation water scarcity and degraded soils, low-income local communities can grow sage for essential oil production using TWW at 60% AWC since under the conditions of the present study such practice resulted in a higher essential oil percentage.

Keywords: irrigation water, water stress, aromatic plants, essential oil yield and percentage, water scarcity, degraded soil, arid region.

INTRODUCTION

Due to the rising global economy and population, rapid urbanization, and climate change, water is becoming a restricted resource in terms of quantity and quality (Forslund et al., 2009). Jordan is one of the most water-scarce countries in the world. It is ranked second among the poorest countries regarding per capita water availability. In addition, Jordan is an arid country with more than 90 percent of the territory receiving less than 200 mm annual precipitation. In this context, reusing treated wastewater is one way to mitigate the adverse impacts of Jordan's already exhausted water resources. The reuse of TWW for irrigation can bring many socioeconomic benefits particularly in marginal areas that has little or no agricultural or industrial value.

Treated wastewater has a high nutritional content, which can help plants grow faster, require less fertilizer, and improve the productivity of poor fertile soils (Al-Lahham et al., 2003). Depending on the source and treatment, treated wastewater may include hazardous levels of salts, heavy metals, or infectious organisms (Qadir et al., 2010; Elsokkary and Abukila, 2014). As a result, the potential for hazardous pollutants from wastewater to enter the agricultural ecosystem would have a negative impact on human health across the food chain (Elsokkary and Abukila, 2014). It is suggested to use low quality wastewater for producing fiber or oil crops to reduce the risk of food contamination when utilizing treated residential sewage effluents for agricultural irrigation (Saber et al., 2002). On the other hand, the reuse of TWW to irrigate cut flowers like roses (Nirit et al., 2006) or for cultivating fragrant plants such as peppermint and corn mint (Zheljazkov and Warman, 2004) could be more acceptable than irrigation food crops with such type of water. However, it is important to evaluate a number of criteria when considering the potential for treated wastewater in irrigation of these plants (Baón et al., 2011). This practice transforms non-conventional low-grade water into productive virtual water for the production of high-value commodities like essential oils (Elsokkary and Abukila, 2014). Indeed, the global essential oils market size was valued at USD 21.79 billion in 2022 and is expected to expand at a rate of 7.9% from 2023 to 2030. This is due to the increasing demand from major end-use industries such as, among others, pharmaceutical uses. Aromatic plants are one of the most significant sources for improvement the economics of agriculture in many countries, and they might be valuable for Jordanian farmers in small-scale farming particularly in rural areas. Many aromatic-medicinal plants are commonly cultivated and consumed in Jordan nowadays. Sage (*Salvia officinalis* L.), the most common aromatic-medicinal herb used for medical purposes, is one of these plants (Bagdat et al., 2017). *Salvia officinalis* L.). Sage plants are very resilient, adaptable and can thrive even in poor soil. Oil production in plants is influenced by a variety of biotic and abiotic factors. Natural and human-induced causes such as water stress have a significant impact on the yield and quality of bioactive compounds in the oil (Biswas et al., 2011). Currently, there is an increasing interest in the reuse of treated municipal wastewater to grow aromatic plants for the production of volatile oils, which are increasingly used for manufacturing cosmetic soaps and perfumes (Hussein et al., 2006) or for treating some human diseases due to their mild features and low side effects (Abu-Darwish et al., 2009). To the best of our knowledge, there is no similar information available regarding the combined effects of treated wastewater and soil moisture content on the growth and essential oil production of aromatic plants. Specifically, the simultaneous impact of treated wastewater as an irrigation source and soil moisture content on essential oil production and yield has yet to be explored. The findings of this study are expected to provide valuable insights for optimizing essential oil production using treated wastewater, particularly under water scarcity conditions in arid regions. Thus, the primary objective of this research was to assess the effects of two distinct irrigation sources (fresh water and treated wastewater) and varying soil moisture levels on the growth performance and essential oil yield of sage plants grown on degraded soil.

MATERIALS AND METHODS

Experimental layout

A pot experiment was conducted under the conditions of a plastic house. Sage plants were transplanted into 30 L plastic pots, each filled with 22 kg of degraded soil brought from the Al-Muwaqqar region (an arid region), with a soil bulk density of approximately 1.63 g cm^{-3}. The key chemical and physical characteristics of this soil are presented in Table 1. The soil was amended with 5% (w/w) quartz sand and 20% (w/w) zeolitic tuff (3 mm) as a soil conditioner. Sage plants were planted at densities of 70 and 50 plants per pot, respectively, grown for 90 and 180 days. Less number of plants was used for the longer growing period to avoid extreme competition among plants grown in each pot. However, growing plants for a longer period could compensate for lower planting density with regard to oil yield as shown below. Sage plants were irrigated with either fresh water or treated wastewater. The primary chemical and microbiological parameters of each water source are presented in Table 2. Soil moisture content was maintained at 60% or 100% of the available water capacity (AWC) throughout the experiment, based on the volumetric soil moisture content at field capacity and permanent wilting point, which were approximately 29.3% and 14.4%, respectively. Irrigation amounts and frequencies were determined by weighing reference pots on a regular basis. The pots were arranged in a completely randomized design with four replications. The treatments included: T1 (fresh water + 100% AWC), T2 (fresh water + 60% AWC), T3 (Treated wastewater + 100% AWC), and T4 (Treated wastewater + 60% AWC).

Table 1. Properties of soil used in the current study

Parameter	Value	Unit
pH (1:1)	7.92	-
EC (1:1)	1.5	dS m^{-1}
Sand	29.3	%
Silt	47.1	
Clay	23.6	
Soil texture	Loam	
Total N	0.065	
Available P	18.3	mg kg^{-1}
Extractable K	716.64	
Soluble Ca	109.37	
Soluble Mg	28.66	
Extractable Na	285	
Soluble Na	149.4	
OM	0.69	%
CaCO3	22.3	
Cd	0	mg l^{-1}
Pb	0.298	
SAR	3.1	-

Table 2. Chemical and microbiological analysis of treated wastewater and fresh water

Parameter	Treated wastewater avg. (min – max)	JISM (2021)	Fresh water avg. (min – max)	JISM (2014)
pH	8.25 (8.11–8.39)	6-9	7.76 (7.60–7.92)	6–9
EC (dS/m)	1.3365 (1.153–1.520)	-	0.9535 (0.857–1.050)	1.7
TN (mg/l)	32.25 (31.8–32.7)	70	0	-
TP (mg/l)	0.5275 (0.134–0.921)	10	0.1075 (0.103–0.112)	6
K (mg/l)	38.61 (21.5–55.72)	-	3.815 (2.28–5.35)	-
Ca (mg/l)	156.9 (152.7–161.1)	-	118.45 (112.2–124.7)	-
Mg (mg/l)	146.15 (136.4–155.9)	-	67.95 (62.2–73.7)	-
Na (mg/l)	120 (115–125)	-	92.5 (90–95)	
Cd (mg/l)	<IDL	0.01	-	-
Pb (mg/l)	0.006	0.2	-	-
SAR	1.635 (1.61–1.66)	9	1.67 (1.64–1.70)	6-12
E. coli (CFU/100 ml)	382.5	1000	-	-
Classification of irrigation water	C3–S1	-	C3–S1	-

Data collection

Soils

Soil was sampled two times during the experiment; i.e. after 90 days and 180 days. Samples were stored in polyethylene bags and transported to the laboratory. Samples were air-dried at room temperature, crushed and sieved using a 2 mm sieve. The soil samples were analyzed for pH, electrical conductivity (EC), OM, $CaCO_3$, nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), cadmium (Cd), lead (Pb), and soil texture. The pH, EC, calcium, and magnesium were determined in 1:1 soil extracts (soil: water ratio). The pH was measured by a pH meter and EC was measured by a conductivity meter. Soil total Nitrogen was estimated by the Kjeldahl digestion method and phosphorus was measured by spectrophotometer according to Olsen method (1954). Extractable potassium was extracted by ammonium acetate method and determined using flame photometer. Soluble calcium and magnesium were determined by complexometric titration with EDTA. Cadmium and Pb were extracted by DTPA method and measured using the atomic absorption spectroscopy. The OM were determined by the Walkley and Black method. The percentage of sand, silt and clay were measured by hydrometer method and soil texture was determined by USDA soil textural triangle.

Water analysis

Treated wastewater and fresh water were stored in galvanized water tanks to be used for irrigation. Water was sampled and analyzed two times during the experiment. Samples were collected in clean and sterilized 1L PE bottles. Samples were analyzed for the pH, EC, TKN, TP, K, Ca, Mg, and SAR according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1998). In addition, the concentrations of Cd and Pb were determined using Atomic Absorption Spectrometer (AAS). A USSL diagram was used to determine irrigation water quality based on sodium adsorption ratio (SAR) and water salinity. The concentration of E. coli in treated wastewater was determined according to IDEXX (2016).

Plant growth parameters and SPAD reading

The height of the plants was measured from soil surface to the tip of the stem. The chlorophyll content of leaves was measured by the SPAD meter. The weight of the fresh and dry leaf biomass was determined at the end of each growing period. The plant growth parameters mentioned above were determined at the end of each growing period.

Plant analysis

Oven-dried leaf materials were grinded to fine powder in an electric grinder for further processing and analyzed for N, P, K, Ca and Mg. One gram of oven-dried leaf materials was subjected to wet digestion in H_2SO_4/H_2O_2 mixture to measure the N concentration by the Kjeldahl method and phosphorus using spectrophotometer. Other elements; i.e. K, Ca and Mg, were estimated using the dry ashing method. Potassium was measured by the flame photometer while calcium and magnesium by the atomic absorption spectrometry. All analyses were performed according to the Association of Official Analytical Chemists (AOAC, 2000).

Extraction of essential oil

Essential oils were extracted from leaf fresh biomass of harvested sage plants by hydrodistillation method using the Clevenger-type apparatus. Hydrodistillation is considered the standard method of essential oils extraction and this method has been widely used for commercial production (Cassel and Vargas, 2006). Fresh leaf biomass was immediately placed in labeled paper bags after each harvest and refrigerated at 5 °C until extraction of essential oils. The fresh leaf biomass was chopped into small pieces as described by Laiq-ur-Rahman et al. (2007). The chopped leaf materials were completely immersed in distilled water inside a round bottom boiling flask, which was boiled on an electric heater for 2 hours. The ratio of fresh leaf biomass (g) and distilled water (ml) was 1:10. After the hydrodistillation process was complete, the essential oils were collected through the graduated distillate receiving tube in the low end of Dean stark trap. Essential oils collected along with aromatic water is drained into a 2 ml tubular glass vial, after a while, the volume of floated oil is measured using a 1ml volumetric pipette graduated 1/100 ml. Essential oil yield extracted from sage fresh leaf biomass was expressed in ml and the percentage of essential oil was expressed as follows:

$$P = \frac{V}{B} \times 100\% \quad (1)$$

where: P – essential oil percentag, V – amount or volume of extracted essential oil (ml), B – eight of fresh leaf biomass.

Statistical analysis

Statistical analysis was separately performed for each growing period using the SAS program. This was because of the differences in planting densities. Data were compared using analysis of variance (ANOVA). Mean separation was carried out using LSD test at 0.05.

RESULTS AND DISCUSSION

Water analysis

The chemical and microbiological composition of treated wastewater did not dramatically change during the experiment as shown by the minimum and maximum values of these parameters (Table 2). Table 2 shows the chemical analysis of treated wastewater and fresh water in comparison with the corresponded values of the Jordanian Standards for irrigation water quality guideline (JISM, 2014) and those for the reclaimed domestic wastewater for industrial crops irrigation (JISM, 2021). The pH of the treated wastewater ranged between 8 to 8.4, which can be considered suitable for irrigation according to the Jordanian Standards for the reclaimed domestic wastewater for industrial crops irrigation (JISM, 2021). While the pH of fresh water ranged between 7.60 and 7.95, which is within the range of the Jordanian Standards for irrigation water quality guideline (JISM, 2014). The EC values ranged between 0.8 to 1 dS/m for the fresh water, which were also compatible with the Jordanian Standards for irrigation water quality guideline. The classification of both water sources was C3-S1, indicating a high salinity hazard but a low sodicity hazard. Total nitrogen (TN) in treated wastewater was lower than the Jordanian Standards for reclaimed domestic wastewater, whereas TP was significantly low in both water sources compared with the Jordanian Standards for reclaimed wastewater and for irrigation water. SAR value for both water sources was significantly lower than the Jordanian Standards for reclaimed wastewater and for irrigation water. With regard to Pb and Cd in treated wastewater, the concentration of these two heavy metals were significantly lower than the permissible limit (JISM, 2021). For the latter reason, the concentration of Cd and Pd was neither determined in soil nor in plant samples.

Effect of water source and soil moisture content on some soil properties

Despite studying the impact of the application of two different water sources at different soil moisture levels on some soil chemical properties for 90 and 180 days, there was no adverse effect in the short run, which was in agreement with Bernstein et al. (2009) and Virga et al. (2020). In addition, the soil analysis data obtained after 90 and 180 days should be discussed with caution. Such data only reflected the soil status at time of sampling because of the continuous uptake of nutrients inherently found in this soil and those added along with irrigation water by a high number of plants grown in each pot or by plants grown for a longer period. This might have prevented the buildup of salts in the irrigated soil. In general, although soil $pH_{1:1}$ was slightly decreased (7.76–7.87) compared with the original pH value (7.92), differences among the different treatments were mostly not significant after 90 as well as 180 days (Table 3). Soil EC was only increased by 0.15 to 0.29 dS m^{-1} compared to the original EC value (1.5 dS m^{-1}); however, similar to pH, differences among the different treatments were mostly not significant (Table 3). Such little increase is not expected to adversely affect neither the vegetative growth nor essential oil yield and supports our hypothesis that using treated wastewater for irrigating sage plants is a potential alternative for fresh water. Moreover, no leaching/drainage was practiced under the conditions of the current study. With regard to soil N, available P, soluble Ca, and soluble Mg, although were slightly higher than those of the original soil, differences were mostly not statistically significant and, thus, no obvious effect of different treatments was observed (Table 3). However, irrigating plants with treated wastewater resulted in significantly higher extractable K concentration after both 90 and 180 days (1224.25 mg kg^{-1} and 1314.36 mg kg^{-1}, respectively) compared with that of fresh water at a soil moisture content of both 60% and 100% of AWC (Table 3). Concerning the soil organic matter, organic matter percentage was not significantly affected by the different treatments neither after 90 days nor after 180 days (data not shown).

The potential accumulation of the heavy metals (particularly Cd and Pb) is not expected. This is because the concentration of Cd in the treated domestic wastewater was even below the instrument detection limit and that of Pb was only 3% of the allowable level (0.2 mg/l) according to the local standards (JISM, 2021). Moreover, most of the heavy metals in calcareous soils (as the soil used in the current experiment) are precipitated as metal carbonate minerals. Three to 6 months are considered as a short-term period. However, it will be worthy to study the medium- and the long-term effect further investigations.

Concerning E. coli, the concentration of E. coli in treated wastewater was much less than that of the local standards (JISM, 2021). In addition, no sprinkler irrigation was used. Although E. coli contamination of soil and pathogen contamination to sage plants are possible, they were not of immediate interest in the current study since sage plants were not grown for direct human consumption. However, we recommend one or more of the following precautions to avoid potential microbial contamination of plants, soil, and workers: less frequent irrigation (under the conditions of the current study, plants were irrigated every 2 to 3 days), the use of sub-surface drip irrigation, avoid daytime irrigation when possible, install sign boards, and/or separate the land from the nearest residential area or vegetable and fruit crops and main roads.

Effect of water source and soil moisture content on some plant growth parameters and SPAD reading after 90 and 180 days of growing period

It was found that plant growth parameters except SPAD readings were significantly affected by water source and soil moisture content after 90 days as shown in Table 4. Table 4 showed that treated wastewater at 100% AWC significantly increased plant height (26.58 cm) and leaf fresh and dry weight (136.65 g and 27.99 g, respectively) compared with the other treatments. This finding confirms that treated wastewater is a potential water source for irrigating sage plants. However, since sage plants were not grown in the current study for direct human consumption (for edible purposes), better vegetative growth is not of utmost importance unless it is directly related to essential oil production (i.e. oil yield and ratio). On the other hand, vegetative growth parameters were significantly reduced by both fresh water and treated wastewater at 60% AWC under the conditions of the current study. This result might indicate that the vegetative growth of sage plants is more sensitive to soil moisture content than water source (or nutrient content of irrigation water) under the conditions of the current study. At the lower soil moisture content, many factors can contribute to the reduced growth parameters. Among others, possible limited root growth and lower soil water potential at 60% AWC can adversely affect water uptake as well as nutrient movement especially those move by diffusion. However, it might not be the case in the open field since soil volume is not as confined as in a pot experiment, which deserves further investigation. Moreover, SPAD readings were not significantly affected by different treatments, which indicated that leaf chlorophyll content of sage plants was not affected by neither water source nor soil moisture content. In addition, only plant height was significantly higher for plants irrigated with treated wastewater at 60% AWC (19.00 cm) compared with those irrigated with fresh water at the same soil moisture content (17.85 cm) after 90 days. (Table 4).

Similar results were obtained after a growing period of 180 days and at a planting density of 50 plants per pot. Both plant height (36.60 cm) and leaf fresh weight (229.09 g) of sage plants were significantly increased when irrigated with treated wastewater at 100% AWC (Table 4). No

Table 3. Effect of water source and soil moisture content on some soil properties

Growing period (days)	Treatments	Soil properties						
		pH	EC (dS/m)	KN %	Available P (mg Kg^{-1})	Extractable K (mg Kg^{-1})	Soluble Ca (mg Kg^{-1})	Soluble Mg (mg Kg^{-1})
90	T1	7.78 ab ± 0.060	1.74a ± 0.11	0.076 ab ± 0.005	27.52a ± 3.97	970.89 b ± 49.63	149.99 ab ± 8.86	35.88 a ± 6.58
	T2	7.76 b ± 0.048	1.68 a ± 0.077	0.069 b ± 0.001	22.00 a ± 4.58	928.32 b ± 35.22	143.88 b ± 11.29	32.11 a ± 3.88
	T3	7.86 a ± 0.048	1.80 a ± 0.11	0.083 a ± 0.009	29.44 a ± 10.56	1224.25 a ± 107.25	160.88 a ± 4.04	39.34 a ± 7.53
	T4	7.82 ab ± 0.044	1.78 a ± 0.051	0.077 ab ± 0.002	27.44a ± 6.22	1122.66 a ± 78.68	157.48 ab ±7.67	36.87a ± 5.25
180	T1	7.78 b ± 0.054	1.72 ab ± 0.032	0.074 a ± 0.004	22.71 a ± 6.50	996.54 b ± 62.51	146.50 a ± 13.11	32.92 a ± 9.81
	T2	7.76 b ± 0.021	1.650 b ± 0.076	0.067 b ± 0.001	20.40 a ± 11.05	915.14 b ± 94.36	143.83 a ± 8.92	27.21 a ± 7.07
	T3	7.87 a ± 0.046	1.76 a ± 0.057	0.078 a ± 0.01	25.29 a ± 14.32	1314.36 a ± 115.97	158.93 a ± 8.80	36.09 a ± 4.71
	T4	7.83 a ± 0.029	1.74 ab ± 0.083	0.072 ab ± 0.004	23.33 a ± 4.24	1183.30 a ± 132.09	152.07 a ± 10.64	34.22 a ± 9.68

Note: means with different letters are significantly different ($p < 0.05$) according to LSD test (n = 4). T1 (fresh water + 100% AWC), T2 (fresh water + 60% AWC), T3 (Treated wastewater + 100% AWC), and T4 (Treated wastewater + 60% AWC). Values are mean ± standard deviation.

significant differences in leaf dry weight were observed between plants irrigated with either fresh water at 100% AWC (60.03 g) or treated wastewater at 100% AWC (62.46 g). Leaf dry weight of plants irrigated with either fresh water at 60% AWC (49.52 g) or treated wastewater at 60% AWC (54.69 g) was significantly decreased (Table 4). It can be observed that the lower soil moisture content, regardless of water source, adversely affected the leaf chlorophyll content of sage plants grown for a longer period (i.e. 180 days). This finding might indicate that SPAD readings become more sensitive to the lower soil moisture content regardless of water source when plants are subjected to this water regime for a longer period compared to those grown for 90 days.

Effect of water source and soil moisture content on essential oil yield and percentage after 90 and 180 days of growing period

Results showed that treatments significantly influenced essential oil yield and percentage of sage plants grown for 90 and 180 days. Figure 1 reveals that the in case of sage plants grown for a shorter period (i.e. 90 days); the effect of different treatments on essential oil yield was not as obvious as that observed for sage plants grown for 180 days (Figure 1). Although plants grown for 90 days resulted in low essential oil yield compared to those grown for a longer period, results showed that essential oil yield was influenced by the different treatments in a similar manner compared to those grown for 180 days. Even though statistically not significant, treated wastewater at 100% AWC resulted in the highest essential oil yield (0.455 ml) in comparison with that of fresh water at 100% AWC (0.42 ml). Nevertheless, essential oil yield extracted from sage plants irrigated with treated wastewater at 100% AWC was significantly higher than that of plants irrigated with treated wastewater and fresh water both at 60% AWC (0.37 and 0.35 ml, respectively) after 90 days (Figure 1). On the other hand, figure 1 showed that only treated wastewater at 100% AWC significantly increased the essential oil yield of sage plants after 180 days. The essential oil yield extracted from sage plants after 180 days was in the following order: treated wastewater at 100% AWC (1.5 ml) > fresh water at 100% AWC (1.17 ml) > treated wastewater at 60% AWC (0.92 ml) = fresh water at 60% AWC (0.80 ml); where no significant differences in essential oil yield were induced by treated wastewater and fresh water both at 60% AWC (Figure 1). Although planting density was lower, essential oil yield extracted from plants grown for 180 days was obviously higher than that of plants grown for 90 days considering that no statistical analysis was run since planting densities were not the same. This observation might lead to the conclusion that growing sage plants for a longer period has more influence on essential oil yield than planting density under the conditions of the current study. Growing plants for a longer period could compensate for lower planting density with regard to oil yield (Figure 1).

Regarding essential oil percentage of sage plants, Figure 2 reveals that the essential oil percentage was also significantly influenced by the treatments. Unexpectedly, the treated wastewater and fresh water both at 60% AWC resulted in

Table 4. Effect of water source and soil moisture content on plant growth parameters and SPAD reading during four harvest

Growing period (days)	Treatments	Plant Height (cm)	SPAD Reading	Leaf Fresh Weight (g)	Leaf Dry Weight (g)
90	T1	23.11 b ± 1.28	9.05 a ± 1.06	128.16 b ± 2.71	25.94 b ± 0.53
	T2	17.85 d ± 1.07	8.26 a ± 1.10	95.93 c ± 3.90	23.58 c ± 1.46
	T3	26.58 a ± 0.84	9.41 a ± 2.24	136.65 a ± 3.26	27.99 a ± 1.44
	T4	19.00 c ± 0.35	8.92 a ± 0.86	100.87 c ± 4.65	23.78 c ± 1.29
180	T1	33.44 b ± 1.47	10.92 b ± 1.42	218.73 b ± 7.91	60.03 a ± 2.78
	T2	27.16 d ± 0.98	8.37 c ± 1.28	150.84 d ± 3.50	49.52 c ± 2.02
	T3	36.60 a ± 0.79	13.78 a ± 0.62	227.09 a ± 3.39	62.46 a ± 3.72
	T4	30.16c ± 0.66	9.14 bc ± 1.80	164.19 c ± 3.80	54.69 b ± 2.60

Note: means with different letters are significantly different ($p < 0.05$) according to LSD test (n = 4). T1 (fresh water + 100% AWC), T2 (fresh water + 60% AWC), T3 (Treated wastewater + 100% AWC), and T4 (Treated wastewater + 60% AWC). Values are mean ± standard deviation.

a significantly higher oil percentage (0.463 and 0.454%, respectively) than that of plants irrigated with these two water sources both at 100% AWC (0.39%) after 90 days (Figure 2).

Results also showed that essential oil percentage was significantly lower for sage plants irrigated with fresh water and treated wastewater both at 100% AWC grown for 90 days. Contrary to the results obtained for the plants grown for 90 days, treated wastewater at 100% AWC resulted in a significantly higher essential oil percentage than only that of the fresh water at 100% AWC.

From a practical and economical point of view, considering that water resources suitable for irrigation are scarce in Jordan, low income local communities in arid regions are encouraged to grow sage plants on degraded soils for the purpose of producing essential oils using treated

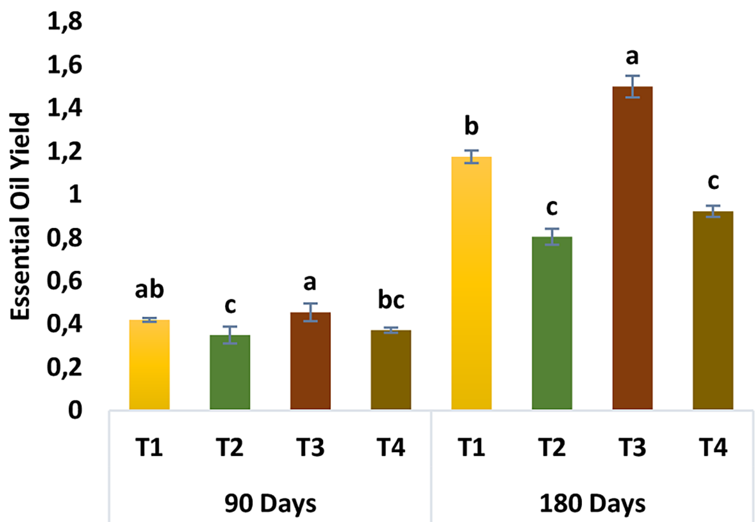

Figure 1. Effects of water source and soil moisture content on essential oil yield after 90 and 180 days of growing period. Means with different letters are significantly different ($p < 0.05$) according to LSD test ($n = 4$). T1 (fresh water + 100% AWC), T2 (fresh water + 60% AWC), T3 (Treated wastewater + 100% AWC), and T4 (Treated wastewater + 60% AWC). Bars are standard deviation. Essential oil yield refers to yield of essential oil (ml) extracted from fresh leaves weight (g)

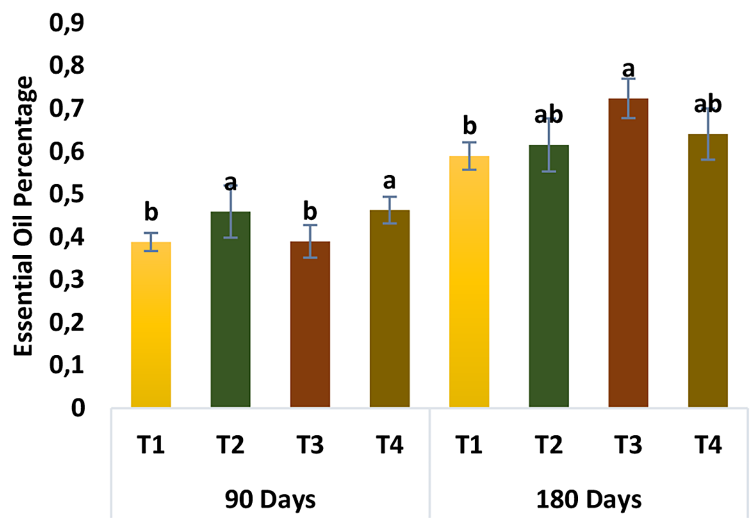

Figure 2. Effects of water source and soil moisture content on essential oil percentage after 90 and 180 days of growing period. Means with different letters are significantly different ($p < 0.05$) according to LSD test ($n = 4$). T1 (fresh water + 100% AWC), T2 (fresh water + 60% AWC), T3 (Treated wastewater + 100% AWC), and T4 (Treated wastewater + 60% AWC). Bars are standard deviation. Essential percentage refers to essential oil yield (ml) per leaf fresh weight (g)

wastewater at 60% AWC. This short period of time and the lower absolute oil yield can be compensated by multiple production cycles irrigated with treated wastewater at 60% of the available water capacity of this type of soils.

Effect of water source and soil moisture content on nutrient content of sage leaf biomass

The analysis of both irrigation water sources revealed significant variations in their chemical composition. The treated wastewater has higher concentrations of TN, P, K, Ca and Mg than fresh water, which generally resulted in higher content of these nutrients in the leaf biomass of sage plants irrigated with treated wastewater particularly at 100% AWC under the conditions of the current study especially for plants grown for 90 days (Table 5). This can be attributed to factors related to the confined soil volume available to sage plants growing in pots as well as to the intensity of rooting. According to Hassan et al. (2020) root system growth was inhibited by soil volume restriction; i.e. the total root length, surface area, dry mass and volume decreased due to this restriction. This observation can be investigated in further studies. The increase in the nutrient content of the leaf biomass of sage plants irrigated with treated wastewater particularly at 100% AWC resulted in an increase in plant height and leaf fresh and dry weight of sage plants regardless of the growing period compared with those irrigated with fresh water at 100% AWC. In addition, correlation analysis revealed that leaf fresh and dry weights were significantly (at $p \leq 0.05$) correlated with the content of N, P, K, Ca, and Mg in the leaf biomass of sage plants (Table 6).

The results also illustrated that growth parameters were adversely affected when sage plants were grown under the conditions of a soil moisture content of 60% AWC particularly for plants irrigated with fresh water for 180 days. For plants grown for shorter periods (i.e. for 90 days) no significant differences in growth parameters were found between fresh water and treated wastewater at 60% AWC. Although statistical analysis was not run among the different growing periods, such response was generally not affected by the planting density, which might indicate that under the conditions of the current study planting density did not affect the response of sage plants to different treatments. However, this observation needs to be further investigated. The reduction in growth parameters of plants maintained at 60% AWC compared to those maintained at 100% AWC can be attributed to different factors. Sage plants grown under a soil moisture content of 60% AWC were possibly subjected to water stress conditions regardless of the differences in planting density and the growing period. However, the soil water depletion fraction for no stress (p) of sage is not available in the literature, which deserves further investigation. Water stress is known to negatively influence the plant's morphological, physiological and chemical processes. In addition, the photosynthesis rate of plants decreases under water stress conditions due to many possible factors such as leaf area reduction, closure or partial closure of the

Table 5. Effect of water source and soil moisture content on nutrient content of sage leaf biomass

Growing period (Days)	Treatment	Nutrient content of sage leaf biomass				
		Leaf N (% Pot^{-1})	Leaf P (mg Pot^{-1})	Leaf K (mg Pot^{-1})	Leaf Ca (mg Pot^{-1})	Leaf Mg (mg Pot^{-1})
90	T1	67.77 b ± 1.79	46.85 ab ± 3.27	551.05 b ± 70.36	236.52 b ± 22.5	163.73 ab ± 35.09
	T2	62.73 b ± 4.91	42.04 b ± 2.29	474.17 b ± 32.32	219.65 b ± 12.37	131.87 b ± 21.6
	T3	75.09 a ± 3.76	52.91 a ± 5.59	671.04 a ± 25.51	320.58 a ± 48.51	204.92 a ± 17.88
	T4	68.33 b ± 5.12	44.49 b ± 7.81	552.62 b ± 61.51	235.10 b ± 20.35	174.89 ab ± 35.92
180	T1	110.03 ab ± 9.62	89.35 b ± 6.45	1176.54 ab ± 110.73	678.40 ab ± 90.43	491.09 ab ± 116.61
	T2	88.00 c ± 3.81	71.05 c ± 6.84	923.10 c ± 70.83	465.40 c ± 43.43	340.58 c ± 37.97
	T3	118.58 a ± 6.96	104.73 a ± 9.43	1369.93 a ± 159.18	849.19 a ± 182.49	580.13 a ± 96.61
	T4	102.41 b ± 5.38	76.63 bc ± 9.78	1093.95 bc ± 169.87	576.91 bc ± 64.28	439.58 bc ± 47.6

Note: means with different letters are significantly different ($p < 0.05$) according to LSD test (n = 4)
T1 (fresh water + 100% AWC), T2 (fresh water + 60% AWC), T3 (Treated wastewater + 100% AWC), and T4 (Treated wastewater + 60% AWC). Values are mean ± standard deviation.

Table 6. Pearson correlation coefficient between leaf fresh and dry weight and some nutrients in the leaf biomass of sage plants

Growing period (days)		N	P	K	Ca	Mg
90	LFW	0.62*	0.67*	0.61*	0.65*	0.50
	LDW	0.88*	0.71*	0.69*	0.61*	0.50
180	LFW	0.79*	0.82*	0.76*	0.77*	0.70*
	LDW	0.94*	0.90*	0.81*	0.77*	0.86*

Note: [1]LFW, [2]LDW: leaf fresh weight and leaf dry weight, respectively. * r values are significant at $p \leq 0.05$.

stomata, reduction in the conductivity of the stomata, lipid peroxidation of membranes, and reduction in the synthesis of protein and chlorophyll. All these factors and consequences can lead to a decrease in the productivity of plants (Asghari et al., 2023). Moreover, the results of the current study are in agreement with those obtained for sweet basil and American basil (Khalid et al., 2006), peppermint (Khorasaninejad et al., 2010) and sage (Sabry et al., 2016). Furthermore, the reduction in growth parameters for plants maintained at a soil moisture content of 60% AWC was greater for plants irrigated with fresh water than those irrigated with treated wastewater especially for plant height after 90 days and for all growth parameters after 180 days. This finding can be attributed to the fact that some nutrients transported in soil via diffusion, particularly phosphorus and potassium, at the 60% AWC might not be easily available for uptake. However, since treated wastewater contains higher concentrations of these nutrients, the slow diffusion of phosphorus and potassium in soil irrigated with treated wastewater at 60% AWC was compensated by the higher amounts of these nutrients added along with the treated wastewater, which might alleviate the possible impact of a lower soil moisture content. Our findings are in agreement with those obtained by Khalid et al. (2006). The latter indicated that the content of N, P and K was adversely affected by drought stress (50% of field capacity) while at 100% field capacity leaves of basil plant had the highest content of N, P and K. On the other hand, Singh-Sangwan et al. (1994) reported that decreased irrigation had an adverse effect on the overall growth of *T. daenensis* (Garden Thyme), which was attributed to the lower availability of sufficient moisture around the root and thus a lesser proliferation of root biomass resulting in lower absorption of nutrients and water leading to production of lower biomass. Moreover, Bahreininejad et al. (2013) found that *Thymus daenensis* was also adversely affected by water deficit. The latter showed that water deficit caused a reduction in fresh and dry weight, plant height and leaf area, in addition to oil yield. The reduction in essential oil yield was due to the reduction in herbage yield Singh-Sangwan et al. (1994). In addition, correlation analysis revealed that essential oil yield was significantly (at $p \leq 0.05$) correlated with leaf fresh and dry weights (Table 7) and the content of N, P, K, Ca, and Mg in the leaf biomass of sage plants (Table 8).

The importance of N, P, and K for essential oil yield were documented by previously published data (Sonmez, 2018; Alhasan et al., 2020). Plant growth, essential oil yield, and other secondary metabolites of aromatic plants grown under different environmental conditions can be adjusted based on the presence and availability of macronutrients. Our results are in agreement with Efendi et al., (2021), who found that the essential oil yield of Kaffir Lime (*Citrus hystrix* DC) was correlated with levels of N, P, and Mg. Our results are also in agreement with Elsokkary and Aboukila (2020) and Amin et al. (2021) who reported that the concentrations of N, P and K in leaves of both basil and oregano plants irrigated by treated wastewater were significantly higher than in those irrigated by fresh water and this increment may be due to the increase in organic matter, macro- and micronutrient in the sewage water. Concerning essential oil percentage, maintaining sage plants at a soil moisture content of 60% AWC resulted in higher essential oil percentage than that obtained at 100% AWC particularly for plants grown for 90 days. However, after 180 days, plants irrigated with treated wastewater at 100% AWC resulted in significantly higher essential oil percentage compared only with those irrigated with fresh water at the same soil moisture content. The increase in essential oil percentage under possible water stress conditions can compensate for the decrease in fresh and dry leaf biomass regardless of the differences in planting

Table 7. Pearson correlation coefficients between leaf fresh and dry weight and essential oil yield and percentage of sage plants

Growing period (days)	Essential oil yield		Essential oil percentage
	LFW	LDW	LFW
90	0.75*	0.50*	-0.72*
180	0.87*	0.68*	0.25

Note: * r values are significant at $p \leq 0.05$.

Table 8. Pearson correlation coefficients between some nutrients in the leaf biomass and essential oil yield of sage plants

Growing period (days)	Essential oil yield				
	N	P	K	Ca	Mg
90	0.67*	0.54*	0.53*	0.37	0.55*
180	0.64*	0.79*	0.77*	0.64*	0.56*

Note: * r values are significant at $p \leq 0.05$.

density. The performance of medicinal and aromatic plants is generally enhanced under stressful conditions by the biosynthesis of secondary metabolites. Such mechanism is one of the defensive mechanism systems for increasing the adaptation of these plants to stressful conditions by mitigating the oxidative stress (Farahani et al., 2009). The results of the current study are in agreement with Govahi et al. (2015), who showed that irrigation after the depletion of 60% of available water (moderate stress) led to the highest increase in essential oil percentage of *S. officinalis* as compared to irrigation after the depletion of 40% (low stress) and 80% (high stress) of available water under field conditions. The same author suggested that the enhanced levels of essential oil percentage under drought stress may be due to the higher density of oil glands. On the contrary, the use of treated wastewater and fresh water both at 100% AWC, particularly after 90 days, resulted in significantly lower percentage of essential oil compared with other treatments regardless of the differences in planting density. In general, the essential oil percentage showed a significant negative correlation mainly with leaf fresh weight (Table 6). According to Simon et al. (1992), water stress increased essential oil accumulation via a higher density of oil glands due to the reduction in leaf area.

CONCLUSIONS

Based on the current study, it can be concluded that treated wastewater offers a viable solution to the problem of water resource scarcity. It serves as an alternative source for irrigating sage plants grown on degraded soil for essential oil production. The results demonstrated that a soil moisture content of 60% AWC increased the essential oil percentage, especially when using treated wastewater for irrigation. Growing such economically valuable plant species under a soil moisture content of 60% AWC could be an effective strategy to conserve water in arid regions. Furthermore, it can be concluded that the reuse of treated wastewater (TWW) for irrigating sage plants, particularly at 60% AWC, can yield significant socioeconomic benefits for impoverished local communities, especially in marginal areas where agricultural or industrial value is otherwise lacking.

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