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RESEARCH ARTICLE
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Essential Oil Production and Sage Growth as Influenced by Treated Wastewater Irrigation and Water Stress

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ABSTRACT

The influence of tap water and treated domestic wastewater (TDWW) at 100% and 60% of the soil available water (SAW) on the percentage and yield of essential oil of *Salvia officinalis* L. grown on a degraded soil in pots inside a plastic house was investigated. Growth parameters of the plants were also evaluated. Sage was cultivated separately at two densities of 90 and 70 plants per pot, respectively, for 45 and 135 days. After 45 days, TDWW at 100% SAW statistically enhanced height of plants and both leaf fresh and dry weights in comparison with other treatment combinations. After 135 days of growth, comparable results were noted, regardless of planting density differences. Furthermore, maintaining 100% SAW for 135 days led to a significant increase in SPAD values, regardless of the irrigation water source. Essential oil yield and percentage were statistically impacted by treatments only during the 45-day growth period. Essential oil yield followed this order: TDWW at 100% SAW (0.35mL) > TDWW at 60% SAW (0.28mL) = tap water at 100% SAW (0.27mL) = tap water at 60% SAW (0.24mL). In contrast, essential oil percentage ranked as follows: TDWW at 60% SAW (0.63%) > tap water at 60% SAW (0.55%) > TDWW at 100% SAW (0.39%) = tap water at 100% SAW (0.38%). Based on the current conditions, cultivating sage for 45 days under 60% SAW with TDWW irrigation presents a promising strategy for low-income communities in arid regions for many reasons: it results in higher essential oil percentage, allows for multiple growing cycles, maximizes return, creates temporal work opportunities mainly for women, and generates associated industry.

Keywords: Sage, Treated Domestic Wastewater, Soil Moisture Content, Essential Oil, Arid Regions

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INTRODUCTION

Jordan is among the most water-deprived nations globally, ranking second in terms of the lowest per capita water availability (Al-Addous et al., 2023). More than 90% of Jordan's territory receives less than 200 mm of rainfall annually, thus it is classified as an arid country (Al-kharabsheh, 2020). Consequently, the treated wastewater reuse has become a crucial strategy for addressing the pressure on the country's limited water resources (Abu-Awwad, 2021).

Utilizing treated wastewater for irrigation offers numerous social and economic advantages, especially in marginal regions where agricultural or industrial activities are minimal. Treated wastewater is known for its high nutritional content, which can accelerate plant growth, reduce the need for fertilizers, and enhance the productivity of low-fertility soil (Al-Lahham et al., 2003). Nevertheless, treated wastewater can also carry harmful levels of salts, heavy metals, and pathogens, depending on its origin and the treatment methods applied (Elsokkary and Abukila, 2014). Consequently, the presence of these pollutants in wastewater poses a risk of contaminating the agricultural ecosystem, with potential adverse effects on human health through the food chain (Hassanain et al., 2021). To minimize the risk of food contamination, it is advisable to irrigate non-edible crops, such as oil and fiber plants, with low-quality wastewater rather than using it for food (Saber et al., 2002).

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Another acceptable approach is applying treated wastewater for the irrigation of ornamental plants and plants grown for non-edible purposes, including cut flowers (Nirit et al., 2006) and aromatic plants such as peppermint (Zheljazkov and Warman, 2004) and sage (Ammari et al., 2025). This strategy effectively converts poor quality, unconventional water sources into virtual water assets, enhancing the production of valuable products, particularly essential oils (Asadzadeh et al., 2023; Ammari et al., 2025). Aromatic plants are considered a significant source for enhancing agricultural economic returns in many countries and marginal areas and hold significant potential for small-scale farmers in rural areas of Jordan. A variety of medicinal and aromatic herbs are cultivated and consumed in Jordan. Among others, sage (*Salvia officinalis* L.), known as "Maramia" in Arabic culture, is one of the commonly used aromatic-medicinal herbs for medicinal reasons (Bagdat et al., 2017). According to Alsanosi (2024), sage belongs to the Lamiaceae family and is popular for its unique aroma, earthy flavor, and notable medicinal qualities. This versatile herb is often used to make herbal teas and infusions for their potential health benefits. It is a perennial plant native to the Mediterranean, but also found in Jordan and Saudi Arabia.

According to Karki (2015) as cited by Riaz et al. (2021), the global trade in botanicals is valued at USD 32.702 billion, with the Asian botanical market accounting for USD 14.505 billion and 6.634 million tons. This represents 44.35% of the world's trade by value and 53.13% by volume. China leads the market with a 1.48% share in the export of medicinal and aromatic plants. The global essential oils market was valued at USD 21.79 billion in 2022 and is projected to grow at a rate of 7.9% from 2023 to 2030 (Baser and Bonello, 2025). This is due to the increasing demand from major end-use industries such as food & beverage, personal care & cosmetics, and aromatherapy. Nayak et al. (2025) concluded that essential oils extracted from some plants offer significant potential in various sectors, including therapeutic, industrial, and technological fields. By incorporating advanced extraction methods, regulatory standards, and AI-driven innovations, their sustainable production and utilization can greatly be enhanced.

Abu-Odeh et al. (2023) concluded that there is a pressing need to explore Jordan's rich variety of naturally occurring medicinal plants and their phytochemicals as potential lead compounds in drug discovery and development, especially after the pandemic of COVID-19. Essential oil production in plants is sensitive to numerous biotic and abiotic influences, with water stress, whether naturally occurring or human-induced, being a significant aspect impacting both the quantity as well as quality of essential oils (Biswas et al., 2011). Lately, there has been a growing interest in utilizing treated municipal wastewater for cultivating aromatic plants, primarily aimed at volatile oil production (Ammari et al., 2025). Ghavam and Afzali (2022) found that irrigating with treated wastewater enhances both the quantity and quality of essential oil produced from *R. × damascena*. Moreover, Asadzadeh et al. (2023) reported that using untreated municipal wastewater for irrigation is an effective approach for maximizing the quantity of essential oil from *M. spicata*, as well as optimizing the yield and quality of essential oil from *R. officinalis*. These oils are highly demanded for their applications in the cosmetic industry, including perfumes and soaps (Hussein et al., 2006), as well as for their therapeutic benefits in managing various human health conditions owing to their gentle nature and modest side-effect profile (Abu-Darwish, 2009). Therefore, the aim of this study was to investigate the impact of irrigating garden sage (*Salvia officinalis* L.) with two different water sources: tap water and treated domestic wastewater (TDWW) at 100% and 60% of the soil available water (SAW) on its growth parameters, essential oil yield, and percentage. Sage plants were separately grown for 45 and 135 days at a planting density of 90 and 70 plants per pot, respectively.

MATERIALS & METHODS

Experimental Design

Sage seedlings were transferred into 30-liter plastic pots that contained 22km of degraded calcareous soil containing 0.69% organic matter. It had a bulk density of about 1630kg m^{-3}. The key soil physical and chemical properties are summarized in Table 1. To improve soil properties, quartz sand (5% by weight) and zeolitic tuff (20% by weight, with particle sizes of 3mm) were incorporated as soil amendments. Sage seedlings were cultivated at 90 and 70 seedlings per pot for growing periods of 45 and 135 days, respectively. Fewer plants were used for the extended growing period to minimize intense competition among plants cultivated in each pot and the possible impact of a confined soil volume. Concerning the two growing periods (45 vs 135 days), we hypothesized that extending the growing period could offset the effects of lower planting density on oil yield. Irrigation was carried out using either tap water or TDWW. Chemical and microbiological properties of both irrigation water sources are detailed in Table 2. Throughout the study, soil water was consistently kept at either 60% or 100% of the SAW. Amount and frequency of irrigation were determined by weighing uncultivated reference pots. Irrigation was practiced when reference pots weighed some target as a proportion of their weight at 100% and 60% of SAW due to water "lost" through evaporation. Soil samples were irregularly taken from cultivated pots after irrigation to make sure that soil moisture content is at either 100% or 60% of SAW.

Reference pots were weighed every 2 to 3 days to adjust the irrigation schedule accordingly (Table 3). The design of the experiment was CRD with four replicates. Treatments were: T1 (tap water at 100% SAW), T2 (tap water at 60% SAW), T3 (TDWW at 100% SAW), and T4 (TDWW at 60% SAW).

Water Analysis

Tap water and treated domestic wastewater were stored separately in galvanized steel tanks throughout the experiment. Water samples were collected twice during the study, using clean, sterilized 1-liter bottles. These samples were analyzed to measure pH, EC, total Kjeldahl N, TP, K, Ca, Mg, and the sodium adsorption ratio (SAR), following the standard methods of the American Public Health Association (APHA, 1998). In addition, concentrations of heavy metals, specifically cadmium and lead, were measured by the AAS. Water quality for irrigation purposes was evaluated using the USSL classification diagram, based on SAR values and salinity levels. Furthermore, the presence of Escherichia coli in the TDWW was assessed following the IDEXX method (2016).

Table 1: Properties of soil used in the current study

Parameters	Unit	Value
$pH_{1:1}$	-	7.92
$EC_{1:1}$	dS m^{-1}	1.5
Sand	%	29.3
Silt	%	47.1
Clay	%	23.6
Soil texture	%	Loam
Total N	%	0.065
Available P	mg kg^{-1}	18.3
Extractable K	mg kg^{-1}	716.64
Soluble Ca	mg kg^{-1}	109.37
Soluble Mg	mg kg^{-1}	28.66
Extractable Na	mg kg^{-1}	285
Soluble Na	mg kg^{-1}	149.4
OM	%	0.69
$CaCO_3$	%	22.3
Cd	mg l^{-1}	0
Pb	mg l^{-1}	0.298
SAR	dimensionless	3.1

Growth Performance and SPAD

Plant height was determined as the distance from soil surface to the top of the stem, with several measurements taken per pot. A SPAD device was used to assess chlorophyll content of leaves. At the end of each growth period, both fresh and dry leaf biomass were recorded. Dry biomass weight was determined by drying a portion of the harvested leaves at 65°C for 48hours.

Plant Analysis

Fresh leaves were dried at 68°C for 48hours to calculate their dry weight. After drying, the leaf material was finely ground using an electric grinder to prepare it for further analysis of N, P, K, Ca, and Mg contents. One gram of the oven-dried leaf powder underwent wet digestion using a mixture of H_2SO_4 and H_2O_2 to measure nitrogen concentration via the Kjeldahl method, while phosphorus content was determined using a spectrophotometer. The concentrations of Mg, K, and Ca were analyzed through dry ash; K was determined with a flame photometer, whereas Ca and Mg were quantified using AAS. All procedures were carried out following the guidelines set by the AOAC (2005).

Essential Oil Extraction

The fresh leaf biomass of harvested sage plants was subjected to hydrodistillation via Clevenger-type apparatus for essential oils extraction. Hydrodistillation is considered the standard technique for essential oil extraction and is commonly applied in commercial production (Cassel and Vargas, 2006). After each harvesting period, the fresh leaves were collected and immediately stored in special labeled paper bags and stored at 5°C until extraction. Prior to distillation, the fresh leaves were finely chopped into smaller segments following the procedure mentioned by Laiq-ur-Rahman et al. (2007). Subsequently, the prepared leaf material was fully submerged in distilled water within a one- to two-liter round-bottom boiling flask that was then heated for 2h. A biomass-to-water ratio of 1:10 (g/ml) was maintained. At the end of the distillation process. The essential oils were gathered using a graduated receiving tube positioned at the base of the Dean-Stark apparatus. The mixture of essential oil and aromatic water was transferred and stored in 2mL capacity tubular glass vials. After allowing the contents to settle, the volume of the separated essential oil was measured by a 1ml volumetric pipette, calibrated to 1/100mL accuracy. Essential oil yield from fresh leaves was recorded (in mL) and essential oil percentage was calculated as follows:

$$\text{Oil percentage} = \frac{\text{Amount or volume of extracted essential oil (ml)}}{\text{Weight of fresh leaf biomass (g)}} \times 100\% \quad (1)$$

Statistical Analysis

For each growing period, statistical analysis was conducted independently by Statistical Analysis System software (SAS 9.4), due to variations in planting densities. Data were evaluated through ANOVA, and mean separation was performed by the LSD test at a 5% significance level.

RESULTS & DISCUSSION

Analysis of Irrigation Water

Chemical and microbiological properties of the TDWW showed minimal fluctuations during the experiment, as reflected by the slight changes in measured parameters (Table 2). The pH levels of both TDWW and tap water were found to be appropriate for irrigation (JISM, 2014, JISM, 2021). Both water sources were classified as C3-S1, denoting an elevated salinity and a low sodicity hazards. The TN content in the TDWW remained below the maximum limit established by JISM (2021), while the total phosphorus (TP) was notably low in both water types when compared to the national regulations. The sodium adsorption ratio (SAR) values for both irrigation waters were well within acceptable limits. As for heavy metals, lead and cadmium in the TDWW were substantially lower than the threshold values specified by JISM (2021). Consequently, Cd and Pb were not examined in either soil or plant samples.

Soil Properties as Influenced by Different Irrigation Water Sources and Soil Moisture

No significant or harmful short-term effects of irrigation water sources or soil moisture levels on the selected soil chemical properties were observed (data not shown). For example, the increase in soil EC was minimal, with an increase of only 0.29dS m^{-1} in comparison with initial EC of 1.5dS m^{-1}. This outcome agreed with the results reported by Bernstein et al. (2009) and Virga et al. (2020). Such a slight increase in salinity is unlikely to impact plant growth or essential oil production negatively, supporting our hypothesis that

TDWW is a viable alternative to fresh water for irrigating sage.

Table 2: Chemical and microbiological analysis of treated wastewater and tap water

Parameter	Treated Domestic Wastewater Avg. (min–max)	JISM (2021)	Tap 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/100mL)	382.5 (354–411)	1000	-	-
Classification of irrigation water	C3-S1	-	C3-S1	-

Table 3: Amount of irrigation water applied to sage plants throughout the experiments

Duration of each experiment (day)	Amount of irrigation (L) and water depth (mm/day)			
	Tap water at 100% AWC	Tap water at 60% AWC	TDWW at 100% AWC	TDWW at 60% AWC
45	14.9 (2.77)	10 (1.86)	15.5 (2.88)	10.3 (1.92)
135	60 (3.72)	38 (2.36)	62 (3.84)	39.5 (2.45)

It should be noted that no leaching practices were implemented during the study. Similar trends were recorded for soil nitrogen, available phosphorus, extractable potassium, and soluble calcium and magnesium, with no significant differences observed among treatments. Furthermore, the risk of Cd and Pb accumulation, was considered minimal, as Cd concentrations in the TDWW were lower than the detection limit, and lead concentrations were only about 3% of the permissible limit, which is 0.2mg L^{-1} (JISM, 2021). Additionally, high calcium carbonate content (22.3% $CaCO_3$) of the calcareous soil used in this study likely facilitated the precipitation of heavy metals as carbonate minerals. Regarding *E. coli*, its concentration in the TDWW was substantially lower than the maximum allowable limits set by JISM (2021). Moreover, irrigation was applied directly to the soil surface without the use of sprinklers, reducing the risk of pathogen transfer. Although there is still a potential for *E. coli* contamination of soil and sage plants, this issue was not a central focus of the study, as sage was not grown for human consumption. Nonetheless, for future field applications, we recommend subsurface drip irrigation to further minimize direct contact between irrigation water, plant surfaces, and field workers.

Plant Growth Parameters and SPAD as Influenced by Different Irrigation Water Sources and Soil Moisture

Irrigation water sources and soil moisture levels significantly influenced plant growth after both 45 and 135 days whereas SPAD readings were only significantly affected after 135 days (Table 4). As presented in Table 4, using TDWW at 100% of the SAW led to a notable increase in sage height and fresh and dry weights of leaves (18.38cm, 106.85g, and 18.46g, respectively) after 45 days in comparison with other treatments. A comparable trend was noted after 135 days, even though planting densities varied between periods. Under the same treatment (TDWW at 100% SAW), plant height (31.85cm) and leaf fresh weight (173.70g) were significantly higher than those recorded for other irrigation treatments (Table 4). While no statistically significant differences in dry weight of leaves were found between seedlings irrigated with tap water at 100% SAW (41.60g) and those with TDWW at 100% SAW (45.11g), TDWW still produced slightly higher dry weights under similar soil moisture status. Such findings suggest that TDWW is a viable alternative irrigation source for sage cultivation. However, given that, sage in this study was not cultivated for human consumption; vegetative growth is regarded as a secondary factor unless it is directly linked to the yield and percentage of essential oils.

Conversely, growth parameters were significantly diminished under 60% of SAW for both tap water and TDWW. This indicates that sage growth under the conditions of this study was more influenced by soil moisture levels than by the type or nutrient content of irrigation water. Several factors may explain the reduced growth observed at lower moisture levels, including limited root development and decreased soil matric potential at 60% SAW, which could hinder water absorption and nutrient mobility, especially for nutrients that move by diffusion. Nonetheless, such limitations may be less severe under open-field conditions, where the volume of soil is not confined, suggesting the need for further investigation. Under 60% SAW, plants irrigated with either tap water or TDWW exhibited significantly reduced plant heights (13.00cm and 13.48cm, respectively) as well as lower leaf fresh weights (64.30g and 66.80g, respectively) and dry weights (12.00g and 12.37g, respectively) (Table 4). Additionally, after 135 days, plants irrigated with TDWW at 60% SAW demonstrated significantly greater plant height (23.10cm) and leaf fresh weight (134.39g) compared to those irrigated with tap water under similar soil moisture condition (21.68cm and 125.97g, respectively) (Table 4).

Interestingly, SPAD, which represents the non-destructive measurement of chlorophyll content, was unaffected by either irrigation water source or soil moisture after 45 days (Table 4). This finding suggests that chlorophyll content was neither a growth-limiting factor nor sensitive to water stress under the conditions of this study. However, further investigation is needed, as no data were available on leaf area or leaf thickness, which could provide additional insights into the relationship between chlorophyll content and plant growth.

Table 4: Effect of irrigation water source and soil moisture on plant growth parameters and SPAD after 45 and 135 days growing period

Growing period (Days)	Treatments	Plant Growth Parameters and SPAD reading			
		Plants Height (cm)	SPAD (dimensionless)	Leaf Fresh Weight (g)	Leaf Dry Weight (g)
45	T1	16.24±0.75b	8.12±0.61a	91.30±4.95b	15.42±2.17b
	T2	13.00±0.60c	7.68±0.85a	64.30±6.87c	12.09±1.04c
	T3	18.37±0.57a	8.73±0.90a	106.85±8.54a	18.46±1.50a
	T4	13.48±0.58c	8.35±0.58a	66.80±3.92c	12.37±1.49c
135	T1	29.06±0.42b	11.02±0.39a	163.97±4.64b	41.60±1.45a
	T2	21.68±0.50d	8.61±0.98b	125.97±3.25d	37.77±1.53b
	T3	31.85±0.58a	12.07±0.89a	173.70±3.86a	45.11±1.85a
	T4	23.10±0.83c	8.35±3.17b	134.39±5.25c	38.40±2.49b

Values (mean±SD) with different letters are significantly different ($p<0.05$) according to LSD test (n = 4). The treatments indicate the following: T1 (tap water + 100% SAW), T2 (tap water + 60% SAW), T3 (TDWW + 100% SAW), and T4 (TDWW + 60% SAW).

After 135 days at 70 seedlings per pot, the effect of TDWW irrigation at 100% SAW on plant height (31.85cm) and fresh weight of leaves (173.70g) was comparable to the trend observed after 45 days (Table 4). Similarly, plants irrigated with TDWW at 60% SAW exhibited statistically greater plant height (23.10cm) and fresh weight of leaves (134.39g) than those irrigated with tap water maintained at similar moisture level (21.68cm and 125.97g, respectively) (Table 4). As for chlorophyll content (SPAD values), maintaining soil moisture at 100% SWC resulted in significantly higher the SPAD readings in plants irrigated with either tap or TDWW (11.02 and 12.07, respectively) compared to plants irrigated at 60% SAW (8.62 and 8.35, respectively). These results indicate that low soil water level, irrespective of the water source, negatively impacted the chlorophyll content of plants grown over the extended 135-day period. This observation suggests that SPAD measurements may become increasingly sensitive to reduced soil moisture when plants are subjected to prolonged exposure to limited water availability under the conditions of this study.

A possible relationship between SPAD and leaf dry weight could help explain these results, as changes in chlorophyll content may reflect the plant's ability to maintain overall photosynthetic activity and growth under water stress conditions.

As mentioned earlier and shown in Table 4, growth parameters were negatively impacted when plants were cultivated at 60% SAW, with the negative effects being more pronounced in plants irrigated with tap water for 135 days. In contrast, for plants grown for 45 days, no statistical differences in growth parameters were detected between those irrigated with tap water and TDWW at the same soil moisture level (60% SAW). Although no statistical comparison was made between the two growing periods due to planting densities, the observed trends suggest that planting density did not substantially influence the plants' responses to the irrigation treatments under the conditions of this study. Nevertheless, this observation warrants further investigation to confirm these preliminary findings. The decrease in growth performance observed in plants kept at 60% SAW, in comparison with those at 100% SAW, can be due to a number of factors. Plants subjected to 60% SAW likely experienced water stress, in spite of planting density or duration of the growth cycle. Nonetheless, the "soil water depletion fraction for no water stress, i.e. p value according to the FAO" specific to *Salvia officinalis* L. is not characterized in the current literature, emphasizing the need for further experimental determination. P-value is critical because it defines the threshold at which soil water availability begins to limit plant transpiration and growth, serving as an essential parameter for optimizing irrigation management strategies in water-scarce environments. Without accurate knowledge of P-value for sage, it is challenging to predict the onset of water stress and its associated physiological, morphological, and biochemical impacts. Morphologically, water stress leads to a reduction in cell expansion and division, resulting in smaller leaf area and shorter shoot length. Physiologically, water stress induces stomatal closure to conserve water, which directly reduces stomatal conductance and subsequently limits the diffusion of CO_2 into the leaf mesophyll, thereby impairing photosynthesis. Additionally, prolonged stomatal closure can lead to decreased transpiration cooling, causing leaf temperatures to rise and exacerbating cellular damage. Biochemically, water stress disrupts the balance of ROS, resulting in oxidative stress manifested through lipid peroxidation of cellular membranes, degradation of proteins, and impairment of photosynthetic pigments such as chlorophylls a and b. Furthermore, water deficit conditions impair the biosynthesis of key metabolites involved in plant defense and energy metabolism. Altogether, these stress-induced changes leads to a substantial decline in photosynthetic efficiency, biomass accumulation, and eventually, overall plant productivity (Asghari et al., 2023; Asargew et al., 2024).

Oil Yield and Percentage as Affected by Irrigation Water Sources and Soil Moisture

Treatments statistically affected oil yield and percentage of plants grown for 45 days. As illustrated in Fig. 1, only the application of TDWW at 100% SAW led to a significant increase in essential oil yield. Oil yield from plants followed this order: (TDWW at 100% SAW (0.35ml) > TDWW at 60% SAW (0.28mL) = tap water at 100% SAW (0.27ml) = tap water at 60% SAW (0.24mL)). No statistical differences in essential oil yield were observed among the plants irrigated with tap water at both 100% and 60% SAW and those irrigated with TDWW at 60% SAW after the 45-day growing period (Fig. 1).

Concerning essential oil %, Fig. 2 shows that treatments also had a significant effect. Unlike the trend observed for oil yield, irrigation with TDWW at 60% SAW produced a statistically higher oil % in comparison with tap water at 60% SAW and other treatments after 45 days. Furthermore, the essential oil percentage was notably lower in seedlings irrigated with either tap or TDWW at

100% SAW after 45 days. Interestingly, for sage plants grown for 135 days, neither oil yield nor percentage was statistically impacted by the irrigation treatments under the conditions of this study (Fig. 1 and 2). Govahi et al. (2015) proposed that the increased in oil percentage under drought stress could be linked to an increased oil glands density. However, since physiological or metabolic parameters were neither measured nor an objective of this study, further related investigation is recommended.

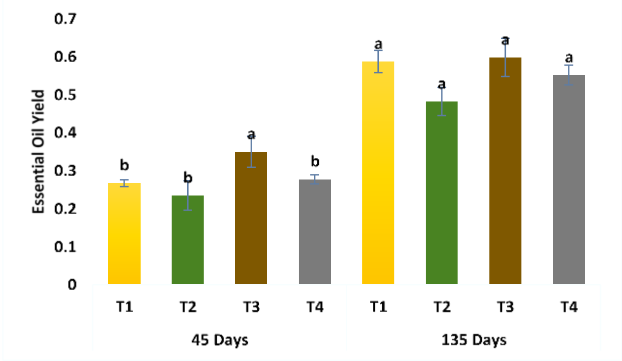

Fig. 1: Effect of source of irrigation water and soil water level on the oil yield of sage plants following 45 and 135 days of growth. Means followed by unlike letters indicate significant differences (p<0.05) based on the LSD (n = 4). The treatments indicate the following: T1 (tap water + 100% SAW), T2 (tap water + 60% SAW), T3 (TDWW + 100% SAW), and T4 (TDWW + 60% SAW). Oil yield represents the volume (mL) of oil extracted per gram of leaf fresh weight. Bars represent standard deviation.

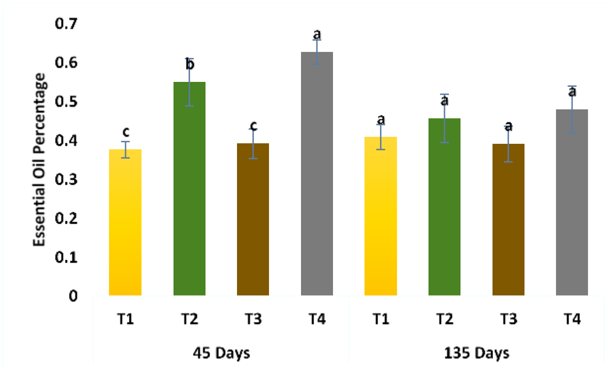

Fig. 2: Effect of source of irrigation water and soil water level on the oil percentage of sage plants following 45 and 135 days of growth. Means followed by unlike letters indicate significant differences (p<0.05) based on the LSD (n = 4). The treatments indicate the following: T1 (tap water + 100% SAW), T2 (tap water + 60% SAW), T3 (TDWW + 100% SAW), and T4 (TDWW + 60% SAW). Oil percentage is expressed as the volume (ml) of oil extracted per gram of leaf fresh weight. Bars represent standard deviation.

After 135 days, the absence of differences in oil yield and percentage, may indicate that sage is not sensitive to the 60% level (a potential water stress) or water source (or the nutrient composition of irrigation water) during this stage of their growth. Sage seems to undergo growth stages that vary in their sensitivity to water stress and water source concerning essential oil production. Despite the lower planting density, the oil yield from sage after 135 days was relatively higher than that from plants harvested after 45 days, although no statistical comparison was made due to the difference in planting densities. This observation indicates that, under the current conditions, growing period may have a greater impact on yield of oil than density of seedlings per pot (Fig. 1). Extending growth period seems to mitigate the negative impact of lower planting density on essential oil production. Longer cultivation allows individual plants to develop larger canopies and more extensive root systems, which enhance photosynthetic activity and secondary metabolite synthesis, including essential oils. Thus, despite the reduced number of plants per unit area, prolonged growth can lead to a cumulative increase in oil yield per plant, partially or fully compensating for the lower population density. This result aligns with the findings reported by Ammari et al. (2025).

Since results indicate that seedlings irrigated with TDWW at 60% SAW produced higher essential oil % compared with the other treatments (Fig. 2), cultivating sage on degraded soils for 45 days under these conditions represents a promising strategy for essential oil production. This approach is recommended as a practical and economically viable solution for low-income communities in arid regions, since under these conditions more essential oil is produced per one gram of fresh leaf biomass. Although the shorter growing period results in a lower absolute essential oil yield per cycle, this can be effectively compensated by implementing multiple successive production cycles. The use of TDWW for irrigation at 60% SAW further supports the sustainability of this approach, especially on degraded soils. Moreover, as indicated in Table 3, the 45-day growth cycle required the least amount of irrigation water, highlighting its efficiency in water-limited environments. Implementing multiple short cycles could thus optimize annual essential oil production while minimizing total water consumption, offering a practical strategy for resource-scarce regions.

Our results agreed with reports published in the literature. Simon et al. (1992) reported that water shortage can enhance oil accumulation by increasing oil glands density, which is associated with decreased leaf area. Additionally, Turtola et al. (2003) suggested that the increase in oil concentration under drought conditions may be contributed to enhanced terpene biosynthesis, as plants prioritize secondary metabolite production when carbon assimilation is limited. Under water stress, reduced cell expansion and growth lead to an accumulation of surplus carbon, which is redirected toward the synthesis of terpenoids via the mevalonate and MEP pathways. As a result, drought-induced metabolic adjustments lead to increased essential oil concentrations, even though overall plant biomass is reduced.

Similarly, Farahani et al. (2008) observed that while the oil yield of balm (*Melissa officinalis* L.) decreased under drought, the essential oil percentage increased. According to Bahreininejad et al. (2013), the increase in oil percentage sufficiently compensated for the yield loss caused by the reduction in herbage yield. Most recently, Mulugeta & Radácsi (2022) reported that under conditions of water stress, the percentage of essential oil typically rises, whereas the overall yield tends to decline. According to Mulugeta & Radácsi (2022), essential oil is stored in the glandular hairs of aromatic plants. Extreme drought led to an increase in glandular hair density in *O. x* *africanum* and *O. americanum*, while the Genovese cultivar exhibited a higher density under moderate drought.

Table 5: Effect of irrigation water source and soil moisture on nutrient content of sage leaf biomass after 45 and 135 days

Growing period (Days)	Treatments	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})
45	T1	49.33±6.72b	27.32±3.65b	291.82±47.02b	131.84±17.3ab	90.91±11.98b
	T2	43.89±3.73b	22.15±1.43b	260.94±32.29b	101.90±15.98b	72.22±13.19c
	T3	60.99±5.31a	34.07±4.11a	384.51±49.29a	183.17±58.03a	129.26±10.08a
	T4	49.96±4.31b	25.27±2.70b	289.10±19.9b	138.77±36.21ab	94.10±13.75b
135	T1	107.62±2.49a	64.91±10.24b	865.98±27.14b	386.53±55.77ab	310.03±44.18ab
	T2	92.68±4.18b	53.76±6.10b	711.16±6.66c	338.56±52.52b	237.11±50.43b
	T3	118.61±7.19a	81.55±7.04a	1044.70±103.69a	399.46±77.5a	344.61±40.61a
	T4	107.02±10.21a	63.02±5.47b	867.16±26.92b	392.16±44.78ab	304.55±52.41ab

Values (Mean±SD) with different letters are significantly different ($p<0.05$) according to LSD test (n = 4). The treatments indicate the following: T1 (tap water + 100% SAW), T2 (tap water + 60% SAW), T3 (Treated wastewater + 100% SAW), and T4 (Treated wastewater + 60% SAW).

Notably, *O. x africanum* demonstrated a glandular hair density that was four times greater than that of *O. basilicum* 'Genovese' and 2.7 times higher than that of *O. americanum*. However, such details will be investigated for sage under the conditions of this study in future trials.

Nutrient Content of Sage Leaf Biomass as Influenced by Different Sources of Irrigation Water and Soil Moisture

Chemical analysis of irrigation water sources showed distinct differences in composition. The TDWW exhibited higher levels of TN, P, K, Ca, and Mg compared to tap water. Consequently, sage irrigated with TDWW, especially at 100% SAW, generally accumulated greater amounts of such nutrients in their leaf biomass. This was specifically the case of N, P, K, and Mg (60.99%, 34.07, 384.51, and 129.26mg kg^{-1}, respectively) for sage plants harvested after 45 days and P and K (81.55 and 1044.70, respectively) for those harvested after 135 days (Table 5). Such results indicate that more nutrients were affected by irrigating sage with TDWW at 100% SAW when plants were grown for 45 days compared with those grown for longer periods (135 days). Such results can be explained by the sage nutrient requirements as affected by its growth stage. However, such detailed information is not available in the literature and deserves a further investigation. Moreover, Hassan et al. (2020) reported that restrictions in soil volume can significantly limit root system expansion, resulting in noticeable reductions in length, surface area, dry mass, and volume of the root system. Limited soil space restricts the natural growth and branching of roots, thereby decreasing the potential of plant to inspect a larger volume of soil for nutrient and water uptake, which might be the case after 135 days. This restriction in root growth often reduced resilience under stress conditions. Moreover, in confined environments like pots, competition among roots for available space and resources further exacerbates these limitations, making soil volume a critical factor in container-grown plant studies.

The increased nutrient content in the sage leaf biomass irrigated with TDWW; particularly at 100% SAW, was associated with increases in height and fresh and dry weights of leaves across both growing periods when compared to plants irrigated with tap water at similar water level.

Moreover, analysis of correlation demonstrated that fresh and dry weights of leaves were statistically correlated ($p \leq 0.05$) with the concentrations of nutrients in the leaf biomass (Table 6). Findings of this research agree with previous findings reported for sweet basil and American basil (Khalid, 2006), peppermint (Khorasaninejad et al., 2011), and sage (Sabry et al., 2016). The decrease in growth performance under 60% SAW was more evident in seedlings irrigated with tap water in comparison with those irrigated with TDWW, with the most noticeable differences observed in height and fresh weight of leaves after 135 days (Table 4). This observation may be linked to the fact that certain nutrients, such as phosphorus and potassium, which are transported in the soil mainly via diffusion, may not be as readily available for uptake at 60% SAW. Nevertheless, because TDWW provides greater amounts of essential elements, the slower diffusion of P and K under 60% SAW may be compensated by the greater nutrient input from the irrigation water itself. This additional supply could help mitigate the adverse impacts associated with lower soil moisture levels. This agreed with the findings of Khalid (2006), who stated that water stress (at 50% of field capacity) led to a decrease in nitrogen, phosphorus and potassium content in basil plants, whereas the highest concentrations of these nutrients were observed in basil leaves grown under full irrigation (100% field capacity).

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
45	LFW[1]	0.74*	0.79*	0.73*	0.44	0.77*
	LDW[2]	0.99*	0.90*	0.78*	0.50	0.81*

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

Regarding essential oil yield, a relationship was observed between growth parameters, nutrient content in the dry leaf biomass, and yield of oil (Table 7 and 8). As mentioned earlier, results demonstrated that sage irrigated with TDWW at 100% SAW achieved statistically greater essential oil yields in comparison with those irrigated with tap water, particularly among plants harvested after 45 days, regardless of planting density variations (Fig. 1). Conversely, when soil water was kept at 60% SAW, no significant variations in essential oil yield were observed between the two irrigation water sources after the 45-day period. These findings agree with those reported by Darvishi et al. (2010), who found that using secondary treated domestic wastewater to irrigate basil plants (*Ocimum basilicum* L.) enhanced both plant growth parameters and essential oil yield. This improvement was mainly attributed to the higher amounts of N, P, and K in the wastewater, which are key nutrients that significantly influence both growth and oil production. Additionally, the correlation analysis shown in Table 7 demonstrated a significant relationship ($p \leq 0.05$) between oil yield and weight of both fresh and dry leaf. Furthermore, after 45 days of growth, essential oil yield was also significantly correlated with the content of N, P, K, Mg, and Ca in leaf tissue of plants (Table 8).

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
45	0.81*	0.76*	-0.75*

*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
45	0.81*	0.72*	0.85*	0.39	0.85*

* r values are significant at $p \leq 0.05$

The crucial role of such nutrients in enhancing oil yield has been stated in earlier studies (Boroom and Grouh, 2012; Chrysargyris et al., 2017; Alhasan et al., 2020). Growth of aromatic species, along with the production of essential oils and the biosynthesis of other secondary metabolites under varying conditions, is significantly impacted by the availability and presence of these key macronutrients. Our findings agree with those of Ammari et al. (2025), who found that essential oil yield in Sage (*Salvia officinalis* L.) was positively correlated with such nutrients. Elsokkary and Aboukila (2020) and Amin (2021) reported similar findings. The later observed that basil and oregano irrigated with TWW exhibited statistically greater leaf N, P and K in comparison with those irrigated with fresh water. This increase was due to the accumulation of organic matter and the enrichment of macro- and micro-nutrients present in treated wastewater, which enhance soil fertility and improve nutrient availability.

In contrast, irrigation with either TDWW or tap water at 100% SAW, particularly after the 45 days, resulted in a statistically lower percentage of oil in comparison with other treatments, irrespective of number of plants per pot. Table 7 illustrates that the oil percentage exhibited a statistical adverse correlation, primarily with fresh weight of leaves.

Conclusion

Our findings indicate that TDWW can serve as an alternative irrigation water for cultivating sage on poor soils in arid regions, helping to address the challenge of limited water availability. The findings showed that maintaining soil moisture at 60% of SAW, particularly when using treated domestic wastewater and during a 45-day growing period, significantly enhanced the essential oil percentage. Cultivating economically valuable aromatic plants like sage under reduced soil moisture conditions (i.e. 60% SAW) may offer a viable strategy for water conservation in arid environments. In conclusion, reusing treated domestic wastewater to irrigate sage plants; especially at 60% SAW, has the potential to bring about considerable socioeconomic advantages for impoverished local societies, particularly in marginal regions where agricultural and industrial prospects are scarce.

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