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The effect of application of effluent water on sage (*Salvia officinalis* L.) yield and quality in lysimeters

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ABSTRACT

Cultivation of medicinal plants with the irrigation of agricultural effluents might be of great importance to save fresh water resources, extend cultivation area and increase economic feasibility. We investigated the effects of saline fish farm effluent water, diluted and gypsum-supplemented effluent, and natural freshwater as a control irrigation in lysimeters. Beside plant growth parameters, macronutrient and sodium content and the amount and composition of essential oil of sage plants were measured. Significant differences among irrigation treatments were found in plant height, SPAD value and essential oil content. Seasonal variation was also observed on plant height, nutrient content of the leaves and the total essential oil content. The essential oil components characterised by the highest levels of availability were detected as α-thujone, camphor, ß-thujone, 1,8-cineol and ledol. Under effluent irrigation, the concentrations of α- and ß-thujone increased slightly; only camphene, trans-sabinole and caryophyllene-oxide changed significantly. The other main components remained stable. Our analysis of the response of sage to the input of effluent provides a reasonable ground for recommending the utilisation of saline effluent water from intensive fish farming in sage production, thus preventing the wastage of valuable water resources.

Keywords: essential oil, growth year effect, lysimeter, salinity, sodium uptake, waste water

Abbreviations: d.m., dry matter; GC-MS, gas chromatography-mass spectroscopy; Irr1, effluent water from intensive fish farm; Irr2, diluted effluent water from intensive fish farm with gypsum; Irr3, Körös-oxbow lake water as control.

INTRODUCTION

Implementation of water reclamation and reuse is a key factor in the pursuit of sustainable water resource management (Roccaro and Verlicchi, 2018). Wastewater reuse can satisfy different needs: irrigation requests, industrial purposes, potable demands and civil uses (Roccaro and Verlicchi, 2018). However, one of the major barriers according to the environmental aspects in implementation of wastewater reuse is salinity (Morris et al., 2021). The salinity of wastewater can come from a variety of sources, such as by-products from distilleries (Galbally et al., 2013), paper mills (Patterson et al., 2008; Quaye et al., 2011), wineries (Hirzel et al., 2017), agro-food, petroleum, textile and leather industries (Castillo-Carvajal et al., 2014; Pounsamy et al., 2019), tannery, drug and petrochemical industries (Srivastava et al., 2021), and even fish farming (Ibadzade et al., 2021; Kolozsvári et al., 2021). In spite of all these, agricultural waste water is used for irrigation in several countries (Qadir et al., 2010).

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Agricultural irrigation using treated wastewater could promote both agriculture and water sustainability, but the primary focus of such a practice should be water recovery and its adoption locally (Ofori et al., 2021). The agricultural costs can be reduced by reusing wastewater for irrigation. This type of water can usually be put to use every season and has no access restrictions (Jiménez, 2006; Khater et al., 2015). Furthermore, severe water scarcity can be alleviated by the use of fish farming effluent water (Castro et al., 2006; Qadir et al., 2010; Soltani, 2017). Intensive fish farming and processing are characterised by the use of large amounts of water, thereby resulting in significant amounts of wastewater (de Melo Ribeiro and Naval, 2019). Water from fish farming has also been shown to be an effective nutrient supply (Haque et al., 2016). The combination of fish farming and agriculture can reduce the need for irrigation of fresh vegetable crops (McMurtry et al., 1997). In our previous research, we found that the effluent of an intensive fish farm in our town provides a constant amount of nutrient-rich water for irrigation, but its use is limited by its sodium and bicarbonate content (Kun et al., 2018a,b; Ibadzade et al., 2020; Kolozsvári et al., 2021). The goal of these experiments was to see if the effluent water from intensive catfish farming could be used to successfully grow herbs despite its salinity.

Saline water irrigation can cause secondary soil salinisation. However, water quality can be improved by the addition of gypsum, which can prevent the accumulation of sodium in the soil. According to the system of water quality classification of Richards (1954), for water characterised by a 0.25–0.75 dS · m^{-1} EC and an 18–26 SAR value or a 0.75–2.25 dS · m^{-1} EC and a 10–18 SAR value, a periodic soil improvement with gypsum is recommended (Richards, 1954; Vyas and Jethoo, 2015). In case of long-term irrigation using water with a high sodium content, it is recommended to dilute the water, if possible, and add Ca-containing materials (Simmons et al., 2009). In the experiments of Kun et al. (2018a), the addition of gypsum to diluted saline effluent water resulted in its improvement, to an extent that characterised it with a resemblance to good-quality river water; and it was classified into a better irrigation water category than the original effluent water according to the Hungarian classification, and the FAO and USDA classification systems as well (Kun et al., 2018a).

The use of saline water for irrigation on various crops, such as quinoa, okra, rice etc., has already been implemented (Azeem et al., 2020; Ibadzade et al., 2020; del Carmen Rodríguez-Hernández et al., 2021), but significantly less research has been done on the irrigation of medical herbs. Aktsoglou et al. (2021) assessed the effect of mild salinity stress during the soilless cultivation of fresh peppermint and spearmint in a floating system on biomass yield, product quality and plant secondary metabolite content. Ozturk et al. (2004) conducted studies to determine the effects of salt stress and water deficiency on some yield components and the essential oil content of lemon balm (*Melissa officinalis* L.). Omer et al. (2013) conducted a field experiment to study the effect of soil salinity and amino acids application on the vegetative growth, flower yield, chemical compositions and essential oil production of *Matricaria recutita* (L.). The research of Sabra et al. (2012) evaluated the physiological and biochemical responses of the three coneflower species *Echinacea purpurea* (L.) Moench, *Echinacea pallida* (Nutt.) Nutt. and *Echinacea angustifolia* (DC.) to NaCl salinity under hydroponic cultivation. Bistgani et al. (2019) aimed to evaluate the effects of saline irrigation, using different NaCl concentrations, on the growth, physiological characteristics, phenolic compounds and antioxidant activity of *Thymus vulgaris* and *T. daenensis*. Cordovilla et al. (2014) published the effect of salinity on thyme (*T. vulgaris*) and lavender (*Lavandula angustifolia*) plants, grown alone and in combination with each other.

The common sage (*Salvia officinalis* L.) is a drought and salt tolerant species and may be used in areas affected by these stresses. Salinity had no detrimental effects on these plants, up to a salinity level of 12.3 dS · m^{-1} (Aslani and Razmjoo, 2018). Similar outcomes were reported by Kulak et al. (2020), who discovered that plant growth was not damaged until the attainment of a concentration of 150 mM, but that above this threshold, salinity adversely affected the development. However, many other studies in the literature completely contradict these results. Göçer et al. (2021) reported decreasing biomass parameters of two *Salvia* species with increasing EC level (salinity solution of NaCl with 1, 2, 3, 4, 5 and 6 dS · m^{-1}). Ben Taarit et al. (2009) also observed a decreased growth (63%) both in the shoot and the root at the above-mentioned threshold (100 mM). Hendawy and Khalid (2005) also found that plant height (PH), as well as fresh and dry leaves' yield, decreased at the salinity level of 2,500 ppm. Moreover, 3,000 ppm sodium chloride was lethal for sage plants. This salinity level is equal with 50 mM sodium chloride. However, this is not an abnormal result, because the adverse effects caused by a level of salinity in excess of the tolerance range of the plant are well-documented in the literature, with multiple studies, delving into the salt effect on the growth of medical plants in particular, agreeing that there is a resultant inhibition. According to Kulak et al. (2020), different salt compounds with increasing concentration affect the vegetative growth and yield of *Salvia officinalis* (L.). Se applied at a concentration of 10 ppm brought about the maximum dry yield in sage (first harvest, 3.72 g · plant^{-1}), and additional Se reduced the influence of salt stress (Yaldiz and Camlica, 2021). $MgCl_2$ had a positive influence on dry herb yield, while the most detrimental effect was observed corresponding to the application of Na_2SO_4. Salt stress significantly affected the chemical composition of leaf essential oil in common sage, too, as demonstrated in the research of Es-sbihi et al. (2021), where a decrease in the essential oil content from 1.2% (control) to 0.4% (NaCl) was observed.

Beside the salinity effects, other environmental factors/stresses such as temperature, humidity, light intensity, supply of water and minerals can influence the amounts of oil components (Akula and Ravishankar, 2011; Verma and Shukla, 2015). Among the macronutrients, nitrogen (Rioba et al., 2015) and phosphorus (Nell et al., 2009) did not affect the content of the total essential oil in sage. However, in some components, the essential oil concentration increased with increasing N level (β-pinene) and the N × P interaction also affected α- and β-thujone accumulation (Rioba et al., 2015). Besides, the climate of the cultivation area and the time of harvest are also important factors in determination of the quantity and quality of essential oil content (Zheljazkov et al., 2012; Hassiotis et al., 2014). Traykova et al. (2019) reported that the application of hydroponic and aeroponic growing systems shortened the period from germination to harvest, enhanced plants' flowering and reflected on the composition of the essential oil. The seasonal variation on the essential oil content was certified by Détár et al. (2020) and Gouvea et al. (2012).

The nitrogen content of sage shoot increased from 2.37% to 2.80% by the addition of 150 kg · ha^{-1} nitrogen and 10 tons · ha^{-1} zeolite. The maximum fresh (13,226.2 kg · ha^{-1}) and dry shoot (3,309.2 kg · ha^{-1}) weights were also measured in the treatment of 150 kg N · ha^{-1} and 10 tons · ha^{-1} zeolite, respectively (Hazrati et al., 2022). Salinity stress can cause nutrient deficiencies or imbalances due to the competitions between Na^+ and K^+ as well as Cl^- and NO_3^-. These nutrient disturbances have a negative effect on plant growth and may change the macronutrients and sodium level of plants (Said-Al Ahl and Omer, 2011).

In our study, three irrigation treatments (with different water qualities) were applied during 2 consecutive years. We monitored the plant physiological parameters, the concentrations of sodium and main macronutrients of leaves, and total essential oil content as well as its composition. The experiment aimed to ascertain the applicability of effluent water irrigation to sage (*Salvia officinalis* L.) production, to determine the level of sodium uptake in leaves and to identify the effect of salinity on the oil composition and its stability.

MATERIALS AND METHODS

Our experiment was carried out at the Lysimeter Station of the Research Center for Irrigation and Water Management (ÖVKI), Institute of Environmental Sciences, Hungarian University of Agriculture and Life Sciences (MATE), in Szarvas, Hungary. The soil type characterising the experimental site was Vertisol (Michéli et al., 2015; Schad, 2016). The soil properties that determine the capability of soils to deliver nutrients are shown in Table 1. Soil samples were taken from soil depths of 0–30 cm and 30–60 cm.

Meteorological characteristics

The experimental area is located in the temperate climatic zone with some Mediterranean effects and usually characterised by high fluctuations of temperatures and extreme water conditions (droughts, floods). The meteorological data for the specific growing seasons were provided by an Agromet Solar automatic meteorological station (Boreas Ltd., Érd, Hungary). These detailed meteorological parameters are described in Figure 1.

Table 1. Soil properties (corresponding to samples drawn from soil depths of 0–30 cm and 30–60 cm) (Szarvas, 2020).

Soil depth (cm)	pH (KCl)	Sludge (%)	All water soluble salts (m · m%$^{-1}$)	Total carbonate content (m · m%$^{-1}$)	Humus (m · m%$^{-1}$)	Nitrite + Nitrate-N (KCl)	P_2O_5 (AL) (ppm)	K_2O (AL) (ppm)	Na (AL) (ppm)
0–30	6.86	70–80	0.08	<0.50	1.87	9.62	706.75	405.67	277.17
30–60	6.36	80	0.08	<0.50	1.95	8.38	394.17	350.75	280.00

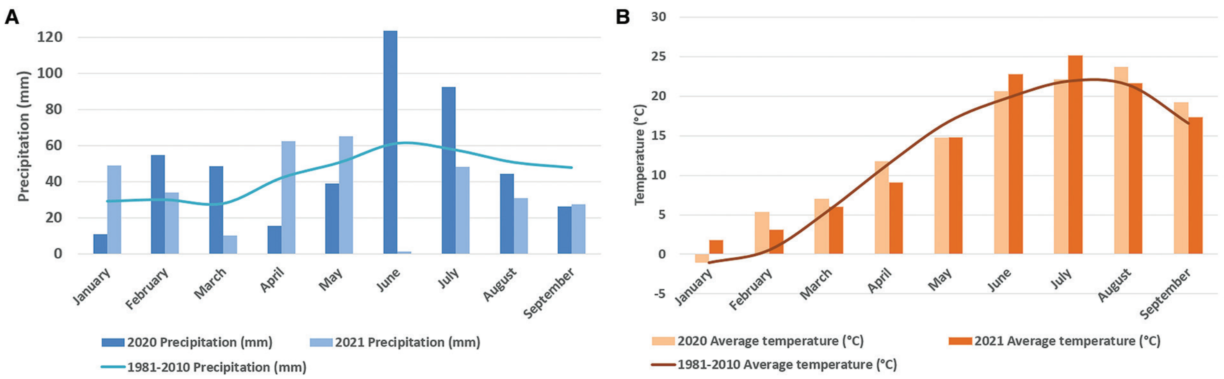

Figure 1. The meteorological data of growing seasons in 2020 and 2021. (A) Sum of monthly precipitation (mm), and (B) the average temperature (°C). The average of 30 years is shown as a reference line.

In 2020, the total yearly precipitation (456.7 mm) was 60.0 mm more than the average for the years 1981–2010; and the average temperature was also higher, with a difference of more than 1°C. In 2021, the precipitation (328.9 mm) was 67.8 mm less than the average for 1981–2010, but the average temperature was 1°C higher. Comparing the experimental data across the various seasons, our results indicated that the precipitation was 127.8 mm more and the average temperature was 0.27 °C higher in 2020 than in 2021. The spring of 2021 (from March to May) was wetter and cooler (sum of precipitation, 137.9 mm; average temperature, 9.94 °C) than the spring of 2020 (103.3 mm and 11.18 °C, respectively). The precipitation fall for June 2020 was 123.8 mm, whereas June 2021 was extremely dry with only 1.2 mm of rainfall.

Plant material and treatments

One-year old uniform sage plants were transplanted into lysimeters (1 m^2 surface) on 22 April 2020. There were four plants in every vessel. We used three irrigation treatments (Irr1, effluent water from an intensive African catfish farm; Irr2, diluted effluent water with gypsum: the effluent water from the intensive African catfish farm was diluted with Körös River water at a ratio of 1:3 and then 0.312 kg · m^{-3} gypsum was added to it; Irr3, Körös-oxbow lake water as irrigated control) in four repetitions. The most important feature of effluent water is the high amount of sodium and bicarbonate content. The detailed quality parameters of the water types Irr1 and Irr3 are listed in Table 2. According to Filep's classification (Filep 1999), the quality of Irr3 is 'impeccable'. Irr2 has the following parameters: EC, 1,073.0 μS · cm^{-1}; NH_4–N content, 10.3 mg · L^{-1}; N content, 13.3 mg · L^{-1}; P content, 1.7 mg · L^{-1}; K content, 5.4 mg · L^{-1}; and Na content, 132.3 mg · L^{-1} (Kolozsvári et al., 2021). The quality of Irr1 is similar to that reported earlier by Karimov (2018). The area was irrigated by micro sprinklers. The irrigation was carried out seven times in both growing seasons,

Table 2. Characteristic properties of irrigation water (2020–2021).

Characteristics of irrigation water	2020		2021	
	Effluent water	Körös-oxbow water	Effluent water	Körös-oxbow water
Temperature of water (in laboratory) (°C)	28.00	24.60	20.00	16.60
pH (in laboratory)	8.18	7.67	7.88	7.67
Specific electric conductivity (20 °C) (μS · cm^{-1})	1,370.00	412.00	1,380.00	329.00
Total alkalinity (p-alkalinity) (mmol · L^{-1})	<0.10	<0.10	<0.10	<0.10
Total alkalinity (m-alkalinity) (mmol · L^{-1})	16.50	3.64	16.70	2.79
Carbonate (mg · L^{-1})	<6.00	<6.00	<6.00	<6.00
Bicarbonate (mg · L^{-1})	1,004.00	222.00	1,016.00	170.00
Ammonium ion (mg · L^{-1})	38.10	1.33	36.10	0.45
Ammonium-N (mg · L^{-1})	29.60	1.04	28.00	0.35
Nitrite ion (mg · L^{-1})	0.33	0.09	0.26	0.10
Nitrite-N (mg · L^{-1})	0.10	0.03	0.08	0.03
Nitrate ion (mg · L^{-1})	<0.44	3.88	<0.44	2.80
Nitrate-N (mg · L^{-1})	<0.10	0.88	<0.10	0.63
Total N (mg · L^{-1})	35.30	2.34	40.60	1.69
Orthophosphate ion (mg · L^{-1})	0.44	0.45	4.88	0.17
Orthophosphate-P (mg · L^{-1})	1.45	0.15	1.59	0.06
Total P (mg · L^{-1})	2.54	0.21	3.68	0.07
Chloride (mg · L^{-1})	33.70	26.80	33.50	20.90
Sulphate (mg · L^{-1})	57.90	27.40	62.40	33.50
Total floating matter (mg · L^{-1})	72.00	3.00	80.00	6.00
Sodium (mg · L^{-1})	282.00	42.60	276.00	22.60
Potassium (mg · L^{-1})	6.72	3.09	6.51	3.00
Calcium (mg · L^{-1})	14.80	34.50	18.80	47.10
Magnesium (mg · L^{-1})	7.53	8.15	8.30	8.57

in sum amounting to 105 mm. The timing of irrigation was adjusted to the lack of natural precipitation, and to the plant observations. The irrigation treatments were carried out on 4 and 29 June, 16 and 30 July, 14 and 19 August and 9 September, 2020. The irrigations were carried out on 30 April, 12 May, 1, 10 and 28 June, 5 July and 11 August, 2021.

Methods of measurements

The measurements and harvests were carried out on 6 May (data not published because of the lack of irrigation treatments), 6 July and 18 September, 2020. In 2021, we could harvest thrice, and thus the measurements were conducted on 19 May, 7 July and 20 September.

We measured the plant properties, such as PH (cm), plant diameter (cm), SPAD value (SPAD 502, Konica Minolta Ltd., Tokyo, Japan), yield: total biomass (g · plant^{-1}), fresh leaves' weight (g · plant^{-1}) and dry leaves' weight (g · plant^{-1}), in 16 replications. The weight of fresh plant parts was measured using a CAS 25 type scale (CAS Co. Ltd, Yangju, South Korea) and the dry leaves' weight was measured using a CAS MWP-1500 device (CAS Co. Ltd). The leaves were dried in a Memmert UFP 800 (Memmert GmbH + Co. KG, Schwabach, Germany) oven at 40°C until the weight was constant.

The leaf essential oil content (mL · 100 g^{-1} dry material) was determined in the Laboratory of the Department of Medicinal and Aromatic Plants of the Hungarian University of Agriculture and Life Sciences, Budapest. Extraction of essential oil from the crumbled plant material was carried out through hydro-distillation, using a Clevenger-type apparatus (according to VII. Hungarian Pharmacopoeia (PhHgVII), Budapest, Hungary) for 2 hr in four replications in each case. The amount is expressed in millilitres per 100 g of dry material (mL · 100 g^{-1} d.m.). GC–MS analysis was carried out using a 6890N GC device (Agilent Technologies, Mulgrave, Australia) equipped with a MS 5975 detector (Agilent Technologies), by using a capillary column (HP-5MS; length, 30 m; id., 250 m; film thickness, 0.25 m), programmed as follows: initial temperature 60 °C, ramp of 3 °C · min^{-1} up to 240 °C. The injector and detector temperatures were 250 °C; helium was used as the carrier gas (constant flow rate, 1 mL · min^{-1}); the split ratio was 30:1; and the ionisation energy was 70 eV. The identification of the constituents was carried out based on the comparison of the retention times with those of authentic samples, comparing the linear retention indices relative to a series of hydrocarbons (C9–C23) using the generalised equation of van den Dool and Kratz (1963), and by using commercial databases (NIST and Wiley) for the mass spectra analysis (Sárosi et al., 2013).

The nutrients (phosphorus and potassium) of sage leaf were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES); however, the sodium was measured by flame atomic absorption spectroscopy (Agilent Technologies, 240FS AA). The nitrogen content of sage leaf (Kjeldahl-nitrogen) was determined by an acidi-alkalimetric type of examination (MSZ EN ISO 5983-2:2009).

Statistical analysis

To evaluate the experimental data, MS Excel 2012 and IBM SPSS 22 (IBM, Armonk, USA) were used, and the figures were generated using the same software applications. The averages and standard deviations were defined by descriptive statistics. The outliers were excluded from further analysis. To determine the effect of the three irrigation treatments on physiological parameters, one-way analyses of variance (ANOVAs) were performed. The Tukey and Games-Howell tests were applied. Pearson's correlation was used to determine the strength of the relationship between the plant and irrigation water parameters. Multivariate analysis of variance (MANOVA) was run to test the effect of treatments on oil compositions. Principal component analysis (PCA) was used to check the difference between the years and among the treatments.

RESULTS

Growth and yield parameters

We analysed sage plants' growing parameters under effluent water irrigation in 2 different meteorological years. The results of the variance analysis for growth traits are shown in Table 3. We detected a significant treatment effect on PH and SPAD value. However, in the case of height, the significance was only detected in the second year between Irr1 and Irr3 (Table 4). The seasonal variation was significant in the case of PH and diameter (Table 3). Our results showed that the harvests in September resulted in the maximum yields in both experimental years (dry leaves' weight in 2020: Irr1, 67.98 ± 17.40 g · plant^{-1}; Irr2, 55.77 ± 18.23 g · plant^{-1}; and Irr3, 46.83 ± 9.47 g · plant^{-1}; in 2021: Irr1, 40.34 ± 12.27 g · plant^{-1}; Irr2, 32.68 ± 7.52 g · plant^{-1}; and Irr3, 32.63 ± 4.90 g · plant^{-1}). In terms of total yield parameters (Figure 2), there were positive significant effects of Irr1 in all cases (biomass, 536.71 ± 119.34 g · plant^{-1}; fresh leaves' weight, 379.63 ± 81.55 g · plant^{-1}; dry leaves' weight, 99.76 ± 18.11 g · plant^{-1}) in 2020. However, in the next drier experimental year, there were no significant differences among the treatments, and thus all applied irrigation water was equally useful in achieving the growth of sage.

The maximum yield of sage was achieved from Irr1 (biomass, 2,146.84 ± 477.37 g · m^{-2}; fresh leaves' weight, 1,518.52 ± 326.18 g · m^{-2}; dry leaves' weight, 399.04 ± 72.44 g · m^{-2}) in 2020. The positive effect of diluted effluent water and gypsum (Irr2) was

Table 3. The results of ANOVA of different traits, showing the SS, df, MS, F test and the level of significance of 16 replications, three treatments and 2 years.

			SS	df	MS	F	Sig.
PH	Treatment	Hypothesis	136.90	2.00	68.45	4.55	0.01
		Error	1,384.20	92.00	15.046		
	Year	Hypothesis	2,847.08	1.00	2,847.08	189.23	0.00
		Error	1,384.20	92.00	15.046		
Plant diameter	Treatment	Hypothesis	142.32	2.00	71.16	2.26	0.11
		Error	2,898.97	92.00	31.511		
	Year	Hypothesis	1,402.86	1.00	1,402.86	44.52	0.00
		Error	2,898.97	92.00	31.511		
SPAD	Treatment	Hypothesis	87.21	2.00	43.60	7.56	0.00
		Error	525.14	91.00	5.771		
	Year	Hypothesis	12.09	1.00	12.09	2.10	0.15
		Error	525.14	91.00	5.771		
Biomass	Treatment	Hypothesis	41,055.81	2.00	20,527.91	1.15	0.32
		Error	1,637,675.00	92.00	17,800.815		
	Year	Hypothesis	21,723.18	1.00	21,723.18	1.22	0.27
		Error	1,637,675.00	92.00	17,800.815		
Fresh leaves' weight	Treatment	Hypothesis	35,391.14	2.00	17,695.57	2.09	0.13
		Error	780,460.39	92.00	8,483.265		
	Year	Hypothesis	1,483.50	1.00	1,483.50	0.17	0.68
		Error	780,460.39	92.00	8,483.265		
Dry leaves' weight	Treatment	Hypothesis	2,234.23	2.00	1,117.12	1.97	0.15
		Error	52,152.62	92.00	566.876		
	Year	Hypothesis	154.28	1.00	154.28	0.27	0.60
		Error	52,152.62	92.00	566.876		
Essential oil content	Treatment	Hypothesis	0.35	2.00	0.17	3.96	0.04
		Error	0.87	20.00	0.044		
	Year	Hypothesis	1.65	1.00	1.65	37.70	0.00
		Error	0.87	20.00	0.044		

ANOVA, analysis of variance; df, degree of freedom; EC, electrical conductivity; MS, mean squares; PH, plant height; Sig., significance; SS, sum of squares; SPAD, SPAD value (Soil Plant Analysis Development) refers to the relative chlorophyll content of leaves.

Table 4. The effects of different water qualities on plant properties of sage in 2020–2021.

Treatment	PH (cm)		Plant diameter (cm)		SPAD value	
	2020	2021	2020	2021	2020	2021
Irr1	38.66 ± 4.06 b	23.83 ± 3.60 a	54.25 ± 4.17 a	43.29 ± 7.47 a	38.46 ± 1.96 b	37.99 ± 2.77 b
Irr2	33.47 ± 4.38 a	25.64 ± 3.20 ab	49.08 ± 4.38 a	43.59 ± 5.34 a	36.98 ± 2.30 ab	32.47 ± 4.55 a
Irr3	37.47 ± 3.75 b	27.46 ± 2.38 b	51.56 ± 2.70 a	47.18 ± 3.73 a	36.31 ± 3.01 a	35.77 ± 1.63 ab

Irr1, effluent water from intensive fish farm; Irr2, diluted effluent water from intensive fish farm with gypsum; Irr3, Körös-oxbow lake water as control; PH, plant height.

The 'a' and 'b' letters mark significant differences among the irrigation treatments at $p = 0.05$.

obtained on the yield in 2021, because the maximum yield was obtained as a result of that treatment (biomass, 1,771.24 ± 596.57 g · m^{-2}; fresh leaves' weight, 1,324.72 ± 396.48 g · m^{-2}; dry leaves' weight, 343.28 ± 92.34 g · m^{-2}), but the differences were not significant (Table 5).

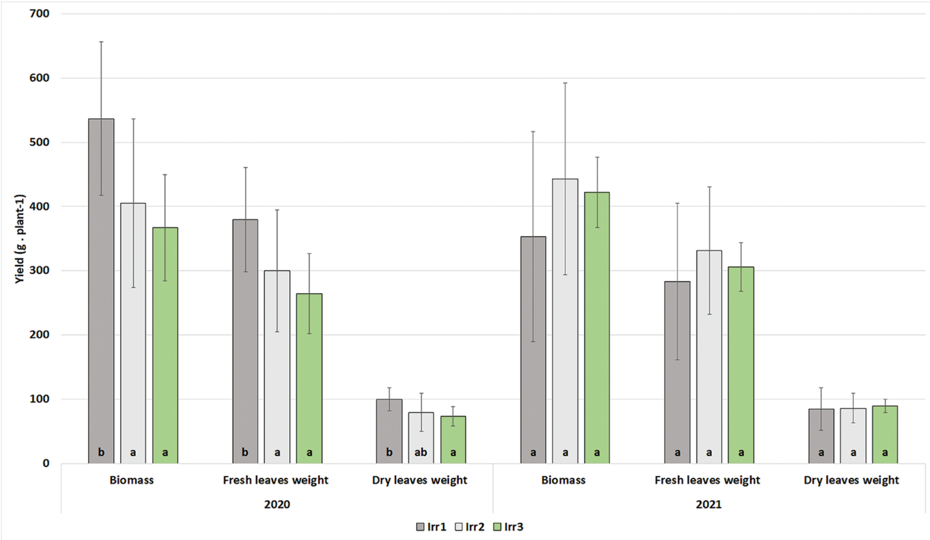

Figure 2. The yield results (biomass and fresh and dry leaves' weight [g · plant^{-1}]) of sage in 2020–2021.

Table 5. Yield (biomass and fresh and dry leaves' weight) results of sage (g · m^{-2}) in 2020–2021.

		Irr1	Irr2	Irr3
2020	Biomass (g · m^{-2})	2,146.84 ± 477.37	1,619.6 ± 525.97	1,467.6 ± 330.84
	Fresh leaves' weight (g · m^{-2})	1,518.52 ± 326.18	1,199.48 ± 380.23	1,055.88 ± 248.94
	Dry leaves' weight (g · m^{-2})	399.04 ± 72.44	316.4 ± 119.14	292.96 ± 61.68
2021	Biomass (g · m^{-2})	1,413.00 ± 653.51	1,771.24 ± 596.57	1,688.76 ± 217.99
	Fresh leaves' weight (g · m^{-2})	1,131.04 ± 487.73	1,324.72 ± 396.48	1,223.75 ± 150.30
	Dry leaves' weight (g · m^{-2})	337.72 ± 132.49	343.28 ± 92.34	357.84 ± 42.82

Irr1, effluent water from intensive fish farm; Irr2, diluted effluent water from intensive fish farm with gypsum; Irr3, Körös-oxbow lake water as control.

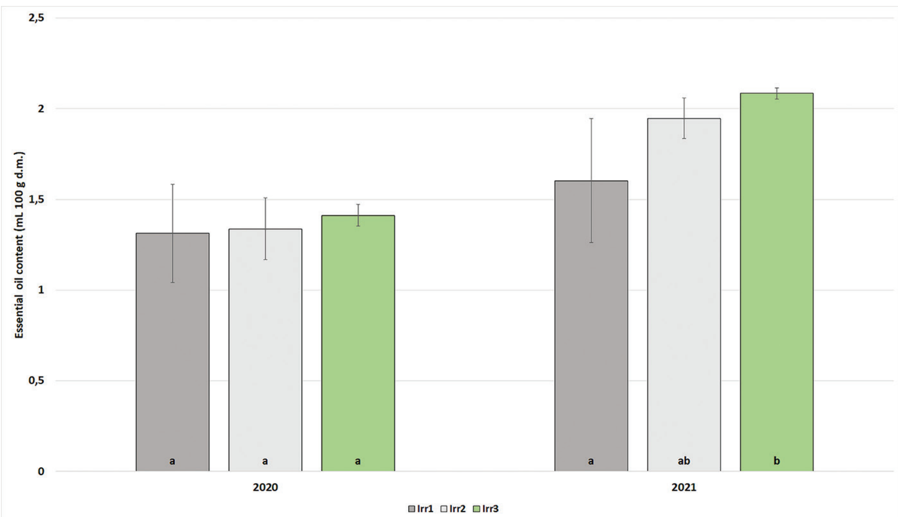

Figure 3. The essential oil content (mL · 100 g^{-1} d.m.) of sage from the September harvest under different irrigation treatments in 2020 and 2021.

Table 6. The components of the essential oil of sage from the September harvest in 2021.

Components of essential oil (%)	Irr1	Irr2	Irr3
Hydrocarbon monoterpenes			
α-Pinene	2.44 ± 0.62 a	2.87 ± 1.12 a	4.04 ± 0.96 a
Camphene*	2.94 ± 0.87 a	3.54 ± 1.01 ab	5.45 ± 1.31 b
Sabinene	0.10 ± 0.04 a	0.10 ± 0.03 a	0.09 ± 0.04 a
β-Pinene	1.26 ± 0.46 a	1.53 ± 0.41	1.80 ± 0.36 a
β-Myrcene	0.65 ± 0.13 a	0.70 ± 0.07 a	0.72 ± 0.07 a
α-Terpinene	0.05 ± 0.10 a	0.06 ± 0.07 a	0.15 ± 0.04 a
Limonene	1.48 ± 0.20 a	1.54 ± 0.10 a	1.80 ± 0.17 a
γ-Terpinene	0.30 ± 0.06a	0.33 ± 0.06 a	0.33 ± 0.08 a
α-Thujene	0.10 ± 0.05 a	0.13 ± 0.04 a	0.15 ± 0.05 a
α-Terpinolene	0.22 ± 0.14 a	0.21 ± 0.06 a	0.28 ± 0.13 a
p-Cymene	0.29 ± 0.07 a	0.27 ± 0.06 a	0.27 ± 0.03 a
Oxygenated monoterpenes			
Trans-sabinene hydrate	0.17 ± 0.05 a	0.15 ± 0.05 a	0.14 ± 0.04 a
Cis-sabinene hydrate	0.16 ± 0.05 a	0.13 ± 0.03 a	0.12 ± 0.03 a
1,8-Cineol	7.72 ± 1.06 a	9.78 ± 0.86 a	8.58 ± 1.65 a
Linalool	0.28 ± 0.06 a	0.27 ± 0.09 a	0.30 ± 0.07 a
α-Thujone	30.37 ± 2.95 a	29.92 ± 3.96 a	26.13 ± 4.95 a
β-Thujone	10.93 ± 1.17 a	8.01 ± 4.52 a	7.94 ± 5.56 a
Iso-3-thujanol	0.16 ± 0.07 a	0.09 ± 0.06 a	0.07 ± 0.07 a
Trans-sabinol*	0.17 ± 0.02 b	0.08 ± 0.06 a	0.10 ± 0.03 ab
Camphor	21.90 ± 3.61 a	23.16 ± 2.17 a	24.03 ± 4.36 a
Isoborneol	2.22 ± 0.15 a	2.14 ± 0.46 a	2.20 ± 0.62 a
Terpinene-4-ol	0.3 ± 0.06 a	0.27 ± 0.08 a	0.26 ± 0.02 a
α-Terpineol	0.15 ± 0.07 a	0.17 ± 0.07 a	0.13 ± 0.01 a
Isobornil-acetate	1.42 ± 0.33 a	1.39 ± 0.26 a	1.97 ± 0.96 a
Trans-sabinil-acetate	0.23 ± 0.09 a	0.18 ± 0.03 a	0.19 ± 0.02 a
Hydrocarbon sesquiterpenes			
β-Caryophyllene	1.82 ± 0.41 a	2.16 ± 0.73 a	0.14 ± 0.48 a
α-Humulene	3.53 ± 1.63 a	3.37 ± 0.38 a	2.80 ± 0.84 a
Oxygenated sesquiterpenes			
Ledol	6.61 ± 2.04 a	5.83 ± 1.14 a	6.77 ± 1.36 a
Caryophyllene-oxide*	0.66 ± 0.24 b	0.25 ± 0.17 a	0.28 ± 0.14 a
Humulene-oxide II	0.51 ± 0.36 a	0.64 ± 0.62 a	1.02 ± 1.20 a

*Significance level ($p = 0.05$).
Irr1, effluent water from intensive fish farm; Irr2, diluted effluent water from intensive fish farm with gypsum; Irr3, Körös-oxbow lake water as control.
The 'a' and 'b' letters mark significant differences among the irrigation treatments at $p = 0.05$.

Essential oil content

We detected significant treatment and year effects in the case of total essential oil content (Table 3). However, the difference between Irr1 (1.60 mL · 100 g^{-1} d.m.) and Irr3 (2.09 mL · 100 g^{-1} d.m.) was certified just in the second experimental year (Figure 3).

Essential oil yield

The influence of less precipitation was observed in 2021 because the essential oil content increased (2020: Irr1, 5.23 mL · m^{-2}; and Irr3, 4.13 mL · m^{-2}; 2021: Irr1, 5.40 mL · m^{-2}; and Irr3, 7.48 mL · m^{-2}) (Table 6 and Figure 3).

Table 7. The nutrient content (N, P, K and Na content, mg · kg^{-1}) of sage leaf from the September harvest in 2020.

Treatment	N (mg · kg^{-1})	P (mg · kg^{-1})	K (mg · kg^{-1})	Na (mg · kg^{-1})
Irr1	2.55 ± 0.16 b	2,950.00 ± 340.49 a	30,357.50 ± 1431.40 a	499.75 ± 42.79 c
Irr2	2.53 ± 0.37 b	2,970.00 ± 677.00 a	28,527.50 ± 1,133.94 a	352.50 ± 42.30 b
Irr3	1.90 ± 0.18 a	2,165.81 ± 596.52 a	29,525.00 ± 1,648.22 a	199.00 ± 8.52 a

Irr1, effluent water from intensive fish farm; Irr2, diluted effluent water from intensive fish farm with gypsum; Irr3, Körös-oxbow lake water as control.
The 'a' and 'b' letters mark significant differences among the irrigation treatments at $p = 0.05$.

Table 8. The nutrient content (N, P, K and Na content, mg · kg^{-1}) of sage leaf from the September harvest in 2021.

Treatment	N (mg · kg^{-1})	P (mg · kg^{-1})	K (mg · kg^{-1})	Na (mg · kg^{-1})
Irr1	2.36 ± 0.36 a	2,750.00 ± 454.83 a	235,02.57 ± 1678.22 a	430.50 ± 29.29 a
Irr2	2.24 ± 0.32 a	3,327.50 ± 334.60 ab	24,905.00 ± 1,103.86 a	397.00 ± 112.42 a
Irr3	2.03 ± 0.09 a	3,650.00 ± 465.47 b	24,695.00 ± 1,278.27 a	374.80 ± 21.31 a

Irr1, effluent water from intensive fish farm; Irr2, diluted effluent water from intensive fish farm with gypsum; Irr3, Körös-oxbow lake water as control.
The 'a' and 'b' letters mark significant differences among the irrigation treatments at $p = 0.05$.

Table 9. The results of Pearson's correlation: the correlation of the total nitrogen content of irrigation water with the plant diameter, shoot length, SPAD value, yield, macronutrient, Na concentration and essential oil content in 2020 and 2021.

Pearson's correlation		Plant diameter	Shoot length	SPAD value	Biomass	Fresh leaves' weight	Dry leaves' weight
Total Nitrogen of irrigation water	2020	0.17	0.14	0.28	0.58**	0.55**	0.50**
	2021	0.33*	0.35*	0.27	0.43**	0.48**	0.40**
	Average of 2020–2021	0.17	0.07	0.27**	0.25*	0.27**	0.32**
		N content of leaves	Phosphorus content of leaves	Potassium content of leaves	Na content of leaves	Essential oil content	
Total Nitrogen of irrigation water	2020	0.62*	−0.17	0.32	0.95**	−0.23	
	2021	0.45	−0.71**	−0.42	0.36	−0.71	
	Average of 2020–2021	0.51*	−0.48*	−0.78	0.28	−0.33	

*Significance level ($p = 0.05$); **significance level ($p = 0.01$).

Based on the overall test of MANOVA of 30 components, it could be ascertained that the treatments had no significant effect on total essential oil content (Wilks'Λ = 0.049, F (18;2) = 0.391; $p = 0.895$). Only camphene, trans-sabinole and caryophyllene-oxide were noted by significant differences among the irrigation treatments (Table 5). Among the various chemical compositions determined as a result of the different irrigation treatments, only camphene, trans-sabinole and caryophyllene-oxide were characterised by significant differences (Table 6).

Nutrient content of sage leaves

The nutrient composition of leaves showed a wide range under effluent irrigation. The phosphorus and potassium content of sage leaf were not characterised by significant differences among the treatments in 2020. However, significantly higher nitrogen (2.55 ± 0.16 mg · kg^{-1}) and sodium content (499.75 ± 42.79 mg · kg^{-1}) were observed (Table 7). In 2021, the potassium and sodium content remained stable, and a decreasing trend in phosphorus content was observed from control to Irr1 treatment (Table 8).

The correlation between the Na content of irrigation water and that of leaves was significant ($r = 0.959$**) in 2020, but not in 2021 ($r = 0.361$). We also detected a negative effect on phosphorus (−0.177 in 2020 and −0.711** in 2021), and a low correlation with potassium (0.300 in 2020 and −0.384 in 2021).

The effect of the total N content of irrigation water

According to Table 9, the strong effect and correlation were proved between the total N content of irrigation water and yield in both years (2020: biomass: $r = 0.58$**;

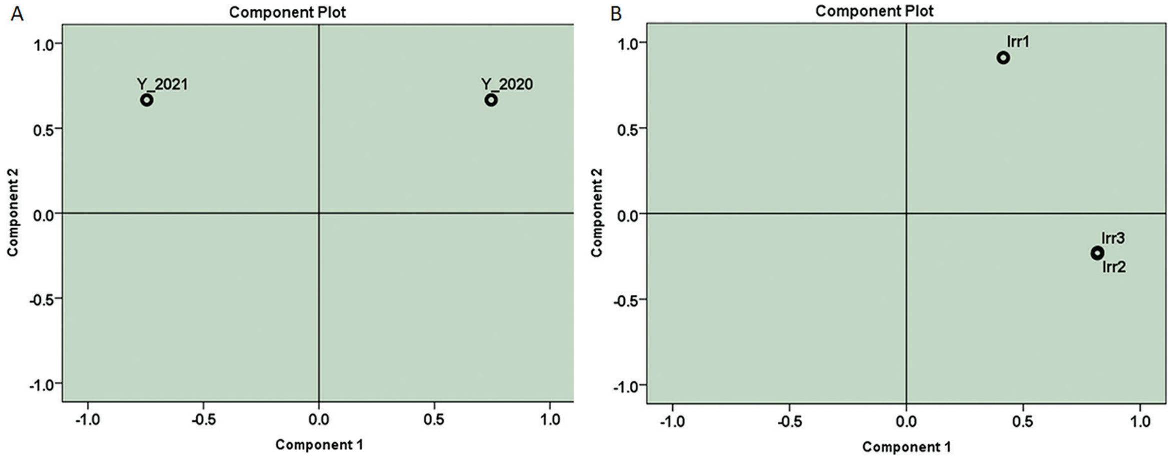

Figure 4. PCAs with two extracted factors. (A) shows the difference between years, where the first component explained 55.56% of the total variance, and the second one 44.44%. (B) represents the difference among treatments, where the first component explained 50.29% of the total variance, and the second one 31.15%. PCAs, principal component analyses.

2021: biomass: 0.43**). The significant negative correlation was observed between the total N content of irrigation water and the essential oil content (r = −0.71**) in 2021.

Principal component analyses (PCAs)

We used PCAs to reduce all examined parameters into correlated factors to identify and visualise the difference between experimental years and among the treatments. For analyses we used factors above 1.0 eigenvalue. Two extracted components are explained, 100% (year) and 81.44% (treatment) of the total variance. As shown in Figure 4, the 2 experimental years (A) and Irr1 treatment (B) are clearly different. Moreover, the Irr2 treatment is not diverged from the control (Irr3).

DISCUSSION

The effluent or reclaimed wastewaters are usually rich in salts (NaCl) and ammonia (Aloui et al., 2009). Accordingly, in our experiment also, the effluent water from the local fish farm was rich in bicarbonate, sodium and ammonia. However, it is characterised by low amounts of other nutrients (Table 2). Usually, aquaculture activities generate wastewater with high quantities of salts (31.1 ± 15 g · L^{-1}) (Srivastava et al., 2021). In our experiment, the sodium content of effluent water was about 276–282 mg · L^{-1} (12 mM), and this is much lower than the amount (830.30 mg · L^{-1}) reported by Castro et al. (2006). Rather, the sodium content observed in the present study aligns to a greater extent with those reported for the water studied by Karimov (2018) and Yeager et al. (2010).

Environmental factors are extremely important in the biosynthesis and accumulation of secondary metabolites (Akula and Ravishankar, 2011; Verma and Shukla, 2015). In terms of salt tolerance of sage, the sodium concentration of effluent water is not a relevant quantity, even if it was irrigated at a level of 105 mm in our experiment. The lethal dose of sodium for the sage plant is about 3,000 ppm in soil (Hendawy and Khalid, 2005). Al-Tabbal et al. (2016) used reverse osmosis (RO) rejected water with an extremely high (1,300 mg · L^{-1}) Na content for sage irrigation. They found that RO rejected water reduced the PH, the number of branches per plant, shoot fresh weight, shoot dry weight and root dry weight by 66%, 79%, 94%, 95% and 85%, respectively, compared to the control irrigation (Al-Tabbal et al., 2016). In the study of Aziz et al. (2013), even the application of 1.7 dS · m^{-1} of NaCl caused 39.91% reductions of sage plant dry biomass in the second cut. When the NaCl concentration was 4.7 dS · m^{-1}, a 137.17% decrease was observed compared to the control (Aziz et al., 2013). Contrastingly, Aslani and Razmjoo (2018) reported no detrimental effects on plants up to a 12.3 dS · m^{-1} salinity level. In our experiment, there was a similarity between the effluent water irrigation's effectiveness on sage biomass production and that of the Körös-oxbow lake water irrigation. This result confirms the conclusion arrived at in the study of Ben Taarit et al. (2009), who did not detect yield decrease until a 25 mmol NaCl concentration.

Hendawy and Khalid (2005) proved that an increasing soil salinity (0–500–1,000–1,500–2,000–2,500 ppm) decreased the fresh and dry flowering herb yields by about 50%. Our results were higher (Table 5) than those of Hendawy and Khalid (2005), because the soil salinity in the present study was only 277.17 ppm corresponding to a soil depth of 0–30 cm, and the sodium content of our irrigation water was also lower with a high total nitrogen content (Table 2). According to Kulak et al. (2020) opinion, treatments under a 150 mM NaCl content do not cause yield damages. We applied irrigation water with a maximum NaCl content of 12 mM, and thus it was favourable for yield (Figure 2 and Table 5). Yaldiz and Camlica (2021) reported that the maximum dry yield of sage was measured from the first harvest (3.72 g · plant^{-1}). Contrastingly, Hendawy and Khalid (2005) reported that the second harvest gave the maximum yield. Our results showed that the September harvests resulted in the maximum yields in both the experimental years (dry leaves' weight: 2020: Irr1, 67.98 ± 17.40 g · plant^{-1}; Irr2, 55.77 ± 18.23 g · plant^{-1}; and Irr3, 46.83 ± 9.47 g · plant^{-1}; 2021: Irr1, 40.34 ± 12.27 g · plant^{-1}; Irr2, 32.68 ± 7.52 g · plant^{-1}; and Irr3, 32.63 ± 4.90 g · plant^{-1}). According to Hazrati et al. (2022), treatment involving the addition of 150 kg N · ha^{-1} + 10 tons · ha^{-1} zeolite resulted in the maximum fresh shoot weight of 1.32 kg · m^{-2}. Our results (Table 9) indicate that we could reach a higher biomass production than Hazrati et al. (2022) with all irrigation treatments. Under control conditions, the Na content of sage leaf was 199.00–374.80 mg · kg^{-1}. This concentration is much lower than that (480 mg · kg^{-1}) involved in the study of Es-sbihi et al. (2021). Under salinity stress, we measured a Na content of 499.75 ± 42.79 mg · kg^{-1} in 2020, and 430.50 ± 29.29 mg · kg^{-1} in 2021. Besides stronger salinity stress, Es-sbihi et al. (2021) mentioned a 1,800 mg · kg^{-1} sodium content; nevertheless, at a lower stress level (170 ppm sodium), Lorente et al. (2022) reported much lower Na^+ concentration. The relationship between essential oil content and salinity stress is not unequivocal. The basic process is the same in many Lamiaceae plants as in glycophyta plants, in that salinity reduces the essential oil yield (Greenway and Munns, 1980). Contrastingly, notable changes in essential oil composition are not induced at low levels of salinity stress (Ben Taarit et al., 2009). Up to 2,500 ppm, an increasing Na^+ concentration in the soil culture increased the oil content (Hendawy and Khalid, 2005). However, Es-sbihi et al. (2021) also confirm that the salt stress decreases the essential oil content. They reported a 33% decrease under 150 mM treatment compared to the control. Our result suggested that irrigation with effluent water decreased the total oil content in the average of 2 years (Irr1, 1.46 mL · 100 g^{-1} d.m.; Irr2, 1.64 mL · 100 g^{-1} d.m.; and Irr3, 1.75 mL · 100 g^{-1} d.m.; Figure 3), especially in 2021 when the precipitation was much lower than in average seasons. This result also highlighted the importance of precipitation as an environmental factor. Missing precipitation causes water shortage, which triggers drought stress easily. Therefore, irrigation is of paramount importance. Mameli et al. (2011) also detected that the amount of irrigation has a significant effect on the oil content of sage. Moreover, it has been discovered that not only the irrigation amount but also the frequency of irrigation has a significant effect on the oil content of sage (Rioba et al., 2015). Plants generally produce higher levels of secondary metabolites under slight drought stress (Selmar and Kleinwächter, 2013). Similarly, Soltanbeigi et al. (2021) observed the highest essential oil content of sage (1.48%) after moderate drought stress. Moreover, additional fertilisers could increase the essential oil content even under serious water shortage compared to the optimal irrigation with NPK application (Soltanbeigi et al., 2021). Nahed et al. (2012) did not detect a significant difference between 80% of effectively irrigated sage plants and the control. However, Nowak et al. (2010) founded an elevated oil content already at 70% of the optimal water supply. The results arrived at in the study of Sonmez and Bayram (2017) also support the proposition that the highest essential oil content is produced corresponding to the prevalence of hot, dry seasons before harvesting.

The relative composition of essential oils varies remarkably with geographical position, climate conditions and several other factors (Şanli and Karadoğan, 2017). Our results are similar to those of Kulak et al. (2020), who indicated that the main components are a-thujone (26.13%–30.37%), camphor (21.90%–24.033%), ß-thujone (7.94%–10.93%), 1,8-cineol (syn: eucalyptol, 7.72%–9.78%) and ledol (5.83%–6.77%). Sonmez and Bayram (2017) detected α-thujone and ß-thujone; Es-sbihi et al. (2021) 1,8-cineol, α-thujone and camphor; and Ben Taarit et al. (2009) viridiflorol, 1,8-cineol, α-thujone and camphor as the main components. On the other hand, according to Aziz et al. (2013), the main components of essential oil of sage are α-thujone (17.28%–21.02%), cisthujone (8.68%–12.64%), camphor (13.45%–18.12%) and 1,8-cineole (7.21%–9.44%). The study of Aziz et al. (2013) demonstrated the highest essential oil percentage and yield vis-à-vis the highest NaCl (4.7 dS · m-^{1}) treatment in comparison with the control plants. We measured a lesser total content of essential oils in the treated plants in 2021, which indicates that the salinity in the effluent water had a stronger influence on oil content, besides the lower precipitation amount. However, significant differences in the main compounds of sage oil were not detected among the treatments. Despite the lower availability of water in the second year (2021), and although there were differences in terms of seasonal variation as well as different water qualities (Figure 4), the oil composition was strongly stable (Table 6). Aziz et al. (2013) found increasing percentages of the major components corresponding to increasing salinity levels, although a decrease in 1,8-cineole was one major observation, too (Aziz et al., 2013).

CONCLUSION

Effluent and gypsum-supplemented effluent water irrigation provide good opportunities to conserve fresh water resources, because the response, especially in terms of the resultant essential oil content, elicited in the sage plant to these methods is similar to that observed corresponding to surface water irrigation. The applied water quality had a significant impact only on the following components, namely camphene, trans-sabinole and caryophyllene-oxide. However, the total essential oil yield was reduced in Irr1 in the second experimental year, compared with the effluent water from intensive catfish farm treatment and Körös-oxbow water irrigation. In consideration of these effects, we can conclude that the effluent water from the African intensive catfish farm, as well as similar effluent water subject to dilution, can be applied towards the irrigated cultivation of sage.

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AUTHOR CONTRIBUTIONS

N. J. V. and M. J. conceived the idea for the study and designed the corresponding experiments. Á. Sz. and N. J. V. performed statistical analysis and drafted the design chart. N. J. V. and Á. K. conducted the field experiments. N. J. V., T. Sz., I. K. and Á. K. collected and analysed the data. Sz. T-S. carried out the essential oil and component analyses. N. J. V. wrote the first draft of the manuscript. N. J. V., T. Sz., Á. Sz., M. J., I. K, Sz. T-S. and Á. K. reviewed the manuscript and prepared its final draft.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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