



## Research article

Sustainable production of *Origanum syriacum* L. using fish effluents improved plant growth, yield, and essential oil compositionFahad Kimera<sup>a</sup>, Hani Sewilam<sup>a,b,\*</sup>, Walid M. Fouad<sup>c</sup>, Ashraf Suloma<sup>d</sup><sup>a</sup> Center for Applied Research on the Environment and Sustainability, The American University in Cairo, Cairo, Egypt<sup>b</sup> Department of Engineering Hydrology, The RWTH Aachen University, Aachen, Germany<sup>c</sup> Department of Biology, The American University in Cairo, Cairo, Egypt<sup>d</sup> Animal Production Department, Faculty of Agriculture, Cairo University, Giza, 12613, Egypt

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## ABSTRACT

The concept of Integrating Aquaculture with Agriculture (IAA) is considered the right path towards achieving sustainable agriculture in semi and arid areas. With the increase of global water scarcity, the double utilization of water for both fish and crop production is gaining more attention since it ensures maximizing the productivity of every unit of water used. This study investigated the effect of fish effluent irrigation on the herbage growth, essential oil content, and composition of *Origanum syriacum* L. The experiment followed a randomized complete design of three irrigation treatments with three replicates, i.e., control with 100% chemical fertilizers (CT), full irrigation with fish effluent (FT), and the mixed treatment (MT) with 50% CT and 50% FT. Study findings showed that FT reached 49 branches/plant, gained maximum plant height (58.8cm), and highest fresh and dry herbage yield reaching 17.76 and 6.722 tons ha<sup>-1</sup>, respectively, in the second cut. Essential oil content reached the maximum in FT at 64.02dm<sup>3</sup> ha<sup>-1</sup> and 143.5dm<sup>3</sup> ha<sup>-1</sup>, while the lowest in CT at 15.95dm<sup>3</sup> ha<sup>-1</sup> and 109.33dm<sup>3</sup> ha<sup>-1</sup> for the first cut and second, respectively. Carvacrol was the main constituent of the excreted essential oil, representing a maximum of 80.87% for FT in the first cut and 74.69% for MT in the second cut. It was closely followed by *p*-Cymene (10.75% - CT, 6.38% - FT) and  $\gamma$ -Terpinene (5.06% - CT, 8.49% - FT) for the first and second cut respectively. The importance of these major chemical components stems from their use in both the food and pharmaceutical industries.

## 1. Introduction

Oregano is a common name that refers to plants that share a particular odor and flavor (Leyva-López et al., 2017; Ortega-Ramirez et al., 2016). These plants are botanically identified under six different families. However, the most obvious ones are the Verbenaceae and Lamiaceae families (Kintzios, 2012; Kintzios, 2004a, b). The genus *Origanum* (Lamiaceae) comprises more than 61 species distributed mainly in Southern Europe, around the Mediterranean regions, and South-west Asia (Kintzios, 2004b). These perennial herbs can be harvested 3–4 times per year with a regrowth cycle of up to 3–4 years. Herbs are widely used in the agro-food, pharmaceutical, perfumery, and cosmetics industries due to their antimicrobial, antifungal, and antioxidative properties (Aziz et al., 2008; Bakkali et al., 2008; Kulisic et al., 2004). However, recent research is focused on the plant's potential to improve human health through pharmaceutical and traditional remedies (Jałoszyński et al., 2008; Radušienė et al., 2008;

Said et al., 2010). Interestingly, across cultures, oregano has been widely used as carminative, diaphoretic, expectorant, emmenagogue, stimulant, and a remedy for headaches, coughs, and toothaches (Ortega-Ramirez et al., 2016). In Mexico, Turkey, and Greece, *Origanum* species are widely used to spice up their food dishes, such as tomato-based sauce, lamb, seafood, and pizza.

Several studies have been conducted on Lamiaceae species, which are commonly known natural antioxidants due to their high amounts of polyphenols and essential oils in their aerial vegetative parts (Dorman et al., 2004; Proestos and Komaitis, 2008). The most commercially cultivated species are *O. vulgare* subsp. *hirtum*, *O. heracleoticum*, *O. onites* and *O. syriacum* (Ortega-Ramirez et al., 2016). These species, in particular, are well known for having a high content of essential oils of up to 2% (Kintzios, 2004a).

Essential oils are defined as complex mixtures of volatile compounds present in aromatic plants characterized by a distinctive odor and high

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resistance to hydrolysis (Dima and Dima, 2015; Shaaban et al., 2012). Oregano is rich in phenolic monoterpenoids, mainly attributed to carvacrol and/thymol as well as  $\gamma$ -terpinene, *p*-cymene, linalool, terpinene-4-ol, and sabinene hydrate (Ciamician, 2000; Kintzios, 2004a; O'Grady and Kerry, 2009).

The quality and commercial value of oregano plants are determined by the content and the composition of their essential oils (Kulisic et al., 2004; Said et al., 2010). Additionally, the quality of the crop is also influenced by the type of genotype, cultivation practices and mostly by the environmental conditions such as the mineral composition of the soil, irrigation patterns, water quality, and fertilizer application (Anwar et al., 2005; Aziz et al., 2008; Edris et al., 2003; Hellal and Mahfouz, 2011; Jajoszyński et al., 2008; Toaima and El-Maghara, 2016a). The high level of both phenolic and flavonoid compounds in oregano is mainly affected by an adequate supply of nutrients and optimal soil moisture. Previous studies demonstrated that higher nitrogen applications in oregano are associated with higher concentrations of monoterpene oxygenated, thymol, and carvacrol, compared to monoterpene hydrocarbons (Aziz et al., 2008; Omer, 1999). In the Mediterranean region, the composition of essential oil in oregano was found to be between 3% to 68% carvacrol (Bernáth, 1997).

To our knowledge, no study had been conducted to investigate the yield and composition of essential oil in *Origanum syriacum* L. cultivated under the Integrated Aquaculture Agriculture (IAA) system. The IAA system applies an on-farm synergy between crops and fish to increase productivity per unit of water used per area. This system's fundamental principle is to recycle and re-use the nutrient-rich aquaculture discharge for integrated sustainable crop production. In their study (Zhang et al., 2011), reported significant benefits of recirculating fish wastewater discharges to combat both water and environmental pollution, which eventually increases water use efficiency in IAA systems. Additionally, a few other studies have also been reported on oregano production under aquaponic systems (Knaus and Palm, 2017; Nozzi et al., 2018; Savidov et al., 2007). Ramírez Sánchez et al. (2011) investigated the productivity of oregano in both aquaponics and hydroponics, and they reported higher fresh and dry yields in the case of aquaponics. Many other studies have been conducted assessing the system's productivity with different crops and reported profound positive results (Dugan et al., 2006; Halwart et al., 2004; Prein, 2002; Suloma and Ogata, 2006). Some authors reported up to 60% net income benefits from savings of fertilizers, fish sales, and pesticide savings by integrating fish with rice and some vegetables (Dey and Prein, 2004; Dey et al., 2005). This research aims at studying the production and growth response of Egyptian oregano (*Origanum syriacum* L.) in sandy soils under different irrigation treatments. This study's main objective is to investigate the effect of fish effluents on the growth, biomass productivity, and essential oil composition of *Origanum syriacum* L. in sandy soils under Egyptian field conditions compared to the control (irrigation with chemical fertilizers).

## 2. Materials and methods

### 2.1. Experiment setup

Field experiments were carried out at the research facility of the Center for Applied Research on the Environment and Sustainability at The American University in Cairo, New Cairo, Egypt (30°01'11.7"N 31°29'59.8"E) in July 2018 and April 2019. During the experimental period, the site recorded a minimum and maximum temperature of 6.9 °C and 36.2 °C, respectively, with an average of 20.5 °C. The average precipitation was 4mm, and radiation of 220.2 W/m<sup>2</sup>.

Oregano seedlings used in this experiment were obtained from the Horticultural Institute at the Agricultural Research Center in Giza-Egypt. The experiment followed a randomized complete block design (RCBD) of three irrigation treatments with three replicates. In the three treatments, compost was added at a rate of 85m<sup>3</sup> ha<sup>-1</sup> two weeks before transplanting.

Control treatment (CT) - freshwater and 100% recommended chemical fertilizers, i.e., nitrogen (ammonium sulphate at 430 kg ha<sup>-1</sup>), phosphorous (single superphosphate at 110 kg ha<sup>-1</sup>), and potassium (potassium sulphate at 60 kg ha<sup>-1</sup>) (FAO, 2005; Kintzios, 2003). Phosphorus was added in full dose during soil preparation. Nitrogen and potassium were added in three equal doses, during planting, at the full establishment, and before flowering. Even though some crops may thrive in some parts of the world without a fertilizer regime, given Egypt's desert climate, fertilizers are added to the soil to ensure plants' survival. Therefore, in this research, the control is the fertilized treatment. In the effluent treatment (FT), crops were only irrigated with tilapia effluent water during all cycles. In the mixed treatment (MT), crops received 50% of CT and 50% FT.

The 45-day old seedlings were transplanted in early August 2018 into isolated experimental beds measuring 2m by 3m, each filled with sandy soil. A total of nine beds were used in this experiment, three beds for each treatment, and each bed consisted of four rows of crops with an intra and inter-row spacing of 30 and 60 cm, respectively, as shown in Figure 1. A single soil sample was obtained and sent for analysis at the Soils, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt. The soil particle size distribution was carried out using the pipette method. Electrical conductivity (EC) values were measured from the soil paste extract; soil pH values were taken from soil suspensions at ratio 1:2.5 as described by Estefan (2013). The available nitrogen in the soil sample was extracted using potassium chloride (KCl) as an extractable solution with the ratio of (5gm soil to 50 ml KCl) and determined using the micro-kjeldahl method. Available potassium was determined using a flame photometer, and the other elements in the soil sample were determined by using inductively coupled plasma (ICP) Spectrometry (model Ultima 2 JY Plasma) (EPA and Thomas, 1991; Soltanpour, 1991). The physical and chemical characteristics of the used soil are presented in Table 1.

All treatments were irrigated at the same rates according to the crop's water needs using drip irrigation lines. Accordingly, the soil moisture content was measured as volumetric water content percentage using a field-scout TDR 350 soil moisture meter from spectrum technologies. All the plots were irrigated every other day to keep the soil moisture level at field capacity. An average volume of 3.83 L/m<sup>2</sup>/day was applied every other day to all plots, and the total irrigation water volume in all the treatments for both cuts was 1023.5 L/m<sup>2</sup>. All plants in all the treatments reached the cutting time (80% flowering) simultaneously.

The fishpond establishment: A trapezoidal plastic-lined pond was established with a depth of 1.5m and a water volume of 6m<sup>3</sup> to cultivate Nile tilapia (*Oreochromis niloticus*). The pond was lined with PVC plastic to avoid seepage and covered with a black net to reduce evaporation losses. Nile tilapia at the stage of fingerlings with a mean weight between 8 – 15 g and total biomass of 8 kg were introduced into the pond in July 2018. Fish were fed with a commercial floating diet supplied by Skretting 10th of Ramadan Rd., El Sharqia, Egypt. Fish feed composition was: 30% crude protein, 5% crude lipid, 6% crude fiber, 13% Ash, and 9% moisture. A sample of 30 fish was measured monthly to monitor fish growth with respect to weight and length and also to regulate the feeding rate. Hence, the fish were fed 3–4 times daily at a feeding rate of 5–8%, depending on their total biomass. Water temperature, pH, and dissolved oxygen were closely monitored daily using an automated Nilebot technologies system supplied by Conative labs, El Basatin, Cairo Governorate. Ammonia, nitrite, and nitrate were checked every week for optimization of Nile Tilapia using API freshwater master test kit. Likewise, for nutrient water analysis, water samples were taken between the two critical crop growth stages of the experimented plant (Oregano), i.e., the vegetative and budding stage. At these stages, the plant gains maximum biomass, and the plant's essential oil levels increase significantly. Therefore, two water samples were collected in the morning before feeding the fish during both stages and immediately sent for chemical analysis at the Agricultural Research Center, Giza, Egypt. Table 2 represents the

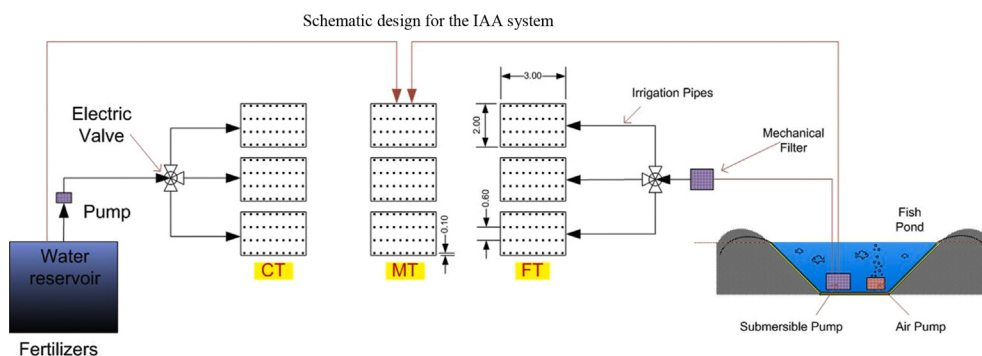


Figure 1. The experimental schematic design of the IAA system used in this experiment.

Table 1. Chemical and physical properties of experimental soil.

Soil Type	pH	EC dS/m	Anions (meq./L)				Cations (meq./L)				N mg/kg	P mg/kg	K mg/kg
			CO <sub>3</sub> <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	Cl <sup>-</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>			
Sand	7.9	2.8	-	1.0	5.2	20.8	9.3	7.2	9.5	1.0	166	1.1	46.5

chemical lab analysis of the fish effluent water used in both FT and MT treatments.

Comparatively, to easily assess and understand the nitrogen nutrient supply for all treatments, the average nutrient levels were calculated. CT variant supplied a total of 9.03 g/m<sup>2</sup> of nitrogen applied throughout the crop's growth during the two cuts, 7.02 g/m<sup>2</sup> of nitrogen in MT and 5.01 g/m<sup>2</sup> in FT.

### 2.2. Determination of relative growth

A total of nine plants were selected at random from each treatment, three plants per replicate, to determine the plant height, in cm as the length of the main stem from the soil surface. The total number of branches per plant was also recorded at different growth stages, i.e., Early Vegetative stage (E.V.S), Late Vegetative stage (L.V.S), Budding stage (B.S), and Full Flowering stage (F.F.S). Two successive cuts for all treatments were done at 80% flowering. Immediately after cutting, fresh weights were obtained using a laboratory-scale balance (PCE-BSK 5100). The plants were then placed in labeled paper bags and dried at 70 °C for three days. Then, plants' dry weights were obtained to estimate the total biomass productivity per treatment. Tilapia fish were harvested at the end of the experiment with an average of 450 g and total biomass of about 300 kg.

### 2.3. Essential oil extraction & gas chromatography-mass spectrometry analysis (GC-MS)

Essential oils were extracted from fresh samples of 100 g weight (stems, leaves, and inflorescences) in triplicates for each treatment. They were hydro distilled over 90 min using 500 ml of distilled water with a fixed extraction time. The distillate was extracted with diethyl ether and dried over anhydrous sodium sulphate. The resulting organic layer of essential oils was concentrated at 30 °C using a vigréux column and then

analyzed. The GC-MS analysis was performed using Agilent Technologies equipped with gas chromatography (7890B) and mass spectrometer detector (5977A) at Central Laboratories Network, National Research Centre, Cairo-Egypt. Samples were diluted with hexane (1:19, v/v) and analyzed using GC equipped with HP-5MS column (30 m × 0.25 mm internal diameter and 0.25 µm film thickness). Samples fractionation were carried out using helium as the carrier gas at a flow rate of 1.0 ml/min at a split ratio of 1:10, injection volume of 1 µl and the following temperature program: 40 °C for 1 min; rising at 4 °C/min to 150 °C and held for 6 min; rising at 4 °C/min to 210 °C and held for 5 min. The injector and detector were held at 280 °C and 220 °C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV; using a spectral range of m/z 40–550 and solvent delay 3 min. The identification of different constituents was determined by comparing the spectrum fragmentation pattern with those stored in Wiley and NIST Mass Spectral Library data.

### 2.4. Statistical analysis

For biomass productivity, data was analyzed based on per square meter productivity. Statistical significance was determined with analysis of variance (ANOVA) using SPSS V22. All the extractions were analyzed in triplicates, and data expressed as mean ± S.D with Duncan test at α = 0.05.

## 3. Results and discussion

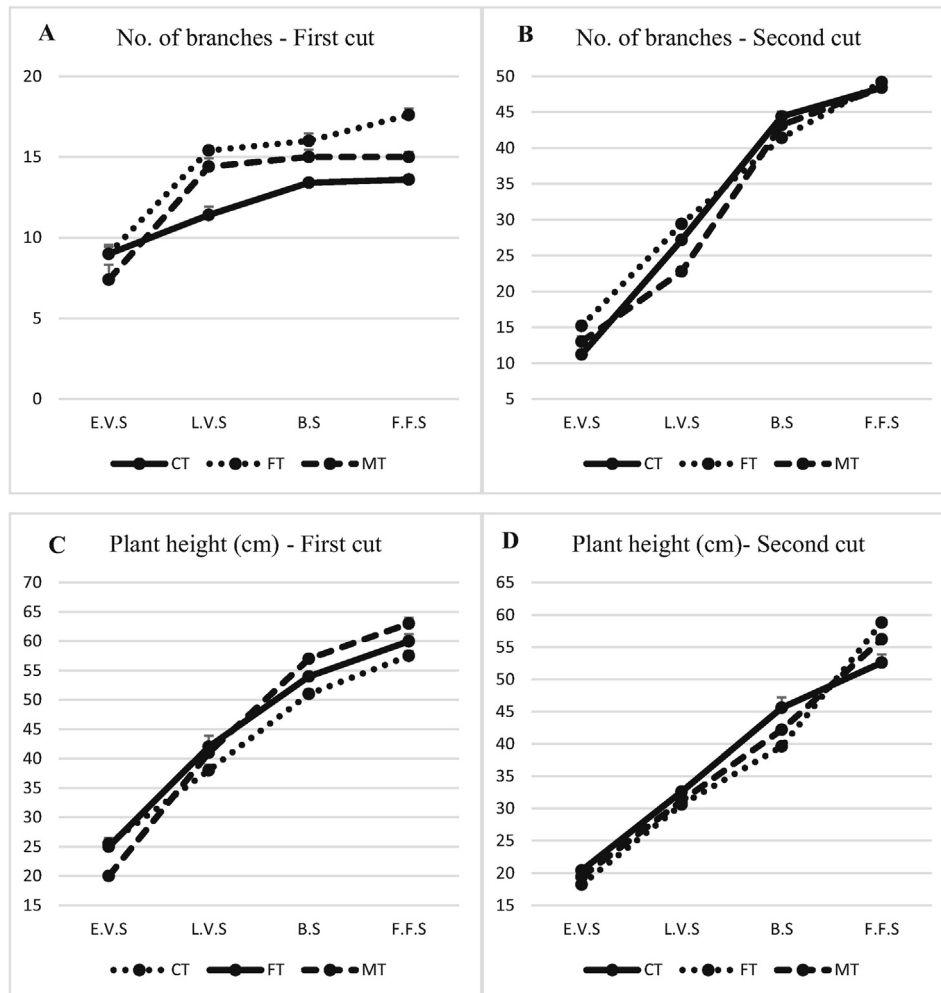
### 3.1. Effect of irrigation treatments on herbage yield

The effect of different irrigation treatments on plant height and the number of branches for *O. syriacum* were assessed, and the results are presented in Figure 2. Results show that different irrigation treatments significantly differed in plants' herbage yield across various growth

Table 2. Chemical analysis of fish effluent water for the two cuts at crop establishment stages.

Water Sample	ppm	pH	EC dS/m	Soluble anions (meq./L)				Soluble cations (meq./L)				NH <sub>4</sub> <sup>+</sup> mg/L	NO <sub>3</sub> <sup>-</sup> mg/L	P mg/L
				CO <sub>3</sub> <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	SO <sub>4</sub> <sup>2-</sup>	Cl <sup>-</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>			
1 <sup>st</sup> Cut	359.5	7.3	0.7	-	0.2	1.2	3.5	1.4	0.5	3.3	-	2.25	13.5	2.3
2 <sup>nd</sup> Cut	370.5	7.5	0.9	-	0.2	1	4.5	1.2	0.5	3.5	0.1	2.35	14.1	2.5
Average	365	7.4	0.8	-	0.2	1.1	4	1.3	0.5	3.4	0.1	2.3	13.8	2.4

## Effect of the different treatments on growth parameters



**Figure 2.** The effect of Irrigation treatments on; A & B: Number of branches in cut 1&2; C & D: plant heights in cut 1&2 at different growth stages. Early Vegetative stage (E.V.S), Late Vegetative stage (L.V.S), Budding stage (B.S) and Full Flowering stage (F.F.S).

stages in both cuts ( $p < 0.05$ ). During the first cut, plants under FT significantly had a higher number of branches at the harvesting stage with 18 branches/plant followed by MT and CT with 15 and 14 branches/plant, respectively ( $p < 0.0001$ ) as shown in Figure 2A. However, for the second cut, all treatments at the harvesting stage, their number of branches more than doubled (48, 48, and 49 branches/plant for MT, CT, and FT, respectively). No statistical significance was noted at the harvesting stage except at E.V.S, L.V.S, and B.S ( $p < 0.05$ ), as shown in Figure 2B. It is expected that cutting would encourage branching, and additionally, the well-established plants in the second cut would have a robust root system that supports vegetative growth (Chimonidou-Pavlidou, 2000; Pal and Mahajan, 2017).

For plant heights in cut one, Figure 2C, results indicate that MT significantly had the highest plant height (63 cm) at the harvesting stage compared to other treatments ( $p < 0.05$ ). However, during the harvesting stage in cut two, FT significantly had a higher plant height (58.8 cm) compared to other treatments ( $p < 0.0001$ ), as shown in Figure 2D.

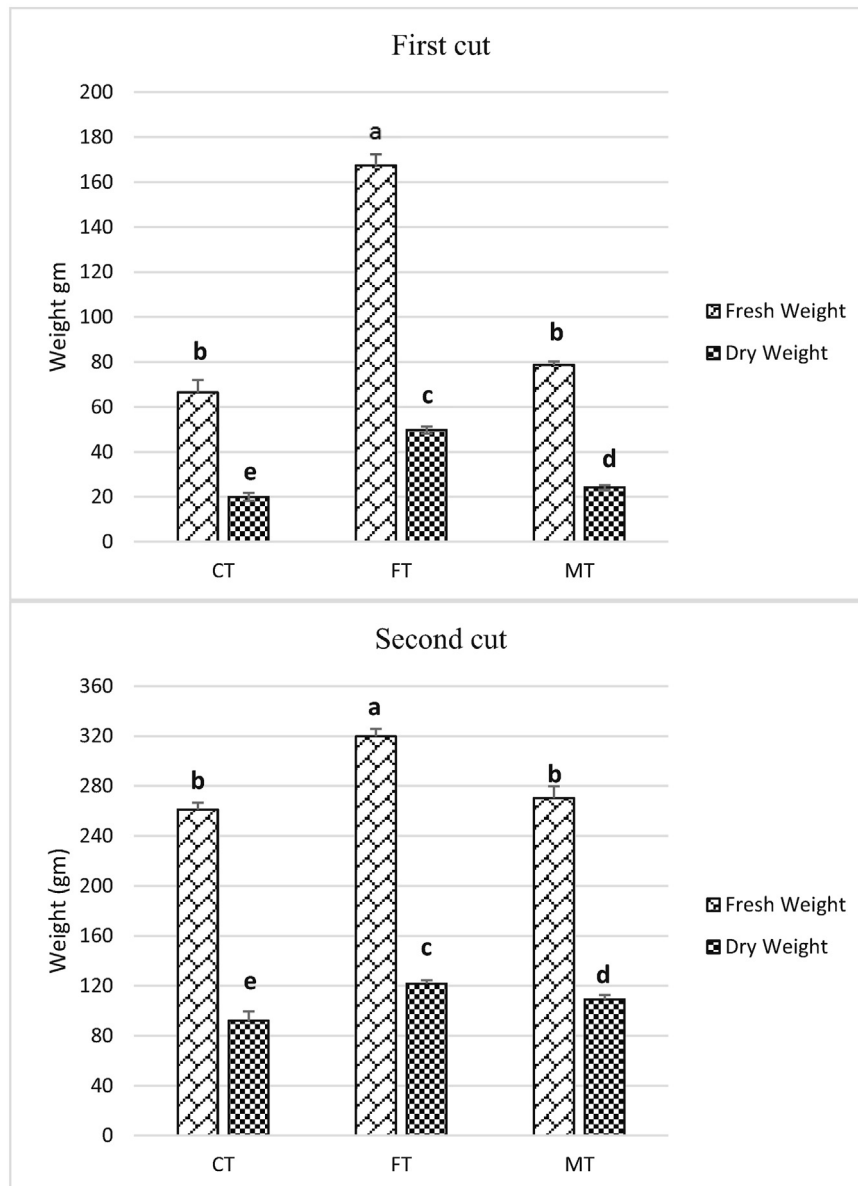
Although the CT had the lowest number of branches and plant height at the harvesting stage, it recorded higher values during E.V.S than other treatments. This might have affected the chemical fertilizers as an activation dose to boost initial crop growth since CT received significant fertilizers at the transplanting time. However, compared to CT, FT significantly had a lower herbage yield at E.V.S ( $p < 0.05$ ). This is due to the longer time taken by organic fertilizers to release nutrients to become more efficient (Naguib, 2011). Generally, there was a gradual increase in

plant height and the number of branches per plant for all the treatments from E.V.S to B.S, which slowed down at F.F.S (Figure 2). Different studies have reported the significant benefit of fish effluents in increasing the herbage yield of crops and the water use efficiency (FAO, 2019; Mariscal-Lagarda et al., 2012; Zajdband, 2011). In his study, Pereira (2002) investigated the effect of fish effluents on lettuce. He reported a significant increase in the plant's shoot biomass while irrigating them with fish effluents.

### 3.2. Effect of irrigation treatments on biomass yield

Regarding the dry and fresh weight production for cut one, results show that FT significantly had higher fresh and dry weights, 167.4 g.plant<sup>-1</sup> and 49.7 g.plant<sup>-1</sup>, respectively, compared to MT and CT ( $p < 0.0001$ ) as shown in Figure 3. Although CT supplied a total of 9.03 g/m<sup>2</sup> of nitrogen and FT supplied 5.01 g/m<sup>2</sup> of nitrogen during the whole season for the two cuts, the continuous application of nitrogen-rich fish effluents could have led to the increase in biomass yield and efficient absorption by plants in the case of FT. Even though (Pereira2002; Seim et al. 1987) reported a range of total nitrogen in fish effluents from 5-10 mg/L, in this study, effluent total nitrogen composition was 4.9 mg/L, slightly lower than the reported range. On the other hand, no significant difference in fresh weight was observed between CT and MT for both cuts. However, in both cut one and two, CT significantly had a lower dry

### Effect of the irrigation treatments on both fresh and dry yield per plant



**Figure 3.** Fresh and dry weight of crops for all three treatments. Data is presented as a means of triplicates for each treatment with Standard Error bars. Bars of the same texture having the same letter above them share no significant difference at  $p > 0.05$  (Duncan Test).

weight (20 g.plant<sup>-1</sup> and 92.9 g.plant<sup>-1</sup> respectively) compared to other treatments ( $p < 0.0001$ ).

The total fresh biomass production ranged from 3.666–9.278 ton ha<sup>-1</sup> for the first cut and 14.5–17.76 ton ha<sup>-1</sup> for the second cut for all the treatments, and the maximum yield was recorded with FT. Our results agree with Baranauskienė et al. (2013); they reported a total fresh weight production ranging from 8.0 to 18.1 ton ha<sup>-1</sup> with *O. vulgare* in Lithuania. In comparison to the two cuts, both fresh and dry weights were significantly different for all treatments at  $t(52) = -14.917$ ,  $p < 0.0001$  and  $t(52) = -19.047$ ,  $p < 0.001$  respectively. The fresh and dry herbage yields using FT for the second cut were 17.76 and 6.722 tons ha<sup>-1</sup>, respectively. This yield is approximately double the yield from the first cut, i.e., 9.278 and 2.777 tons ha<sup>-1</sup> for fresh and dry weight, respectively.

On the other hand, the herbage yield of CT increased by almost four folds from the first cut to the second cut, i.e., 3.666 and 1.111 tons ha<sup>-1</sup> in the first cut and 14.5 and 5.111 tons ha<sup>-1</sup> in the second cut for fresh and dry weights respectively. These, however, were still significantly lower

yields compared to the other treatments. MT recorded 15.012 and 6.058 tons ha<sup>-1</sup> for fresh and dry weights, respectively.

Kintzios (2012) reported that oregano production could reach 2.5–3.5 tons ha<sup>-1</sup> of dry weight with about two cuts annually. This study revealed that irrigating *O. syriacum* with FT can double the reported yields, although MT also recorded promising results. This could mainly be attributed to the higher and continuous levels of nitrogen fertilization from the fish effluents. A study conducted on oregano plants by Ahl et al. 2009 and Omer, 1999 revealed a positive correlation between essential oil content and the available nitrogen in the soil. Other authors have also reported considerably high yields of produce while using fish effluents as an irrigation source for different crops such as rice and maize (Abdul-Rahman et al., 2011; Danaher et al., 2016; Neori et al., 2004). Hussain and Al-Jaloud (1995) reported more than 100% yields while experimenting with aquaculture effluents compared to well water with wheat crops (Wesley et al., 2000).

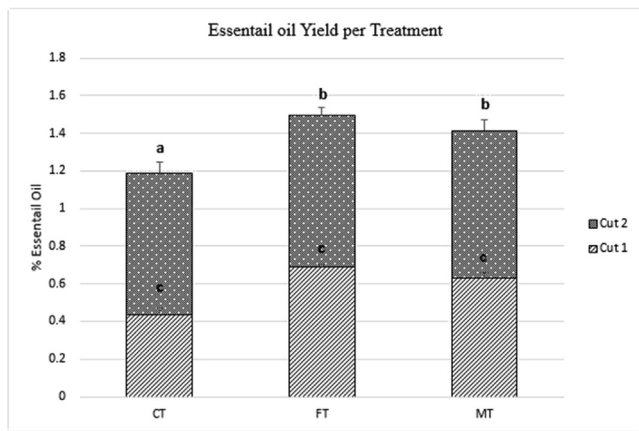


Figure 4. The percentage yield of essential oil for each treatment extracted from a 100gm sample basis. Values are expressed as means of the three triplicates. Bars of the same letter above them share no significant difference at  $p > 0.05$  (Duncan Test).

Furthermore, Limbu et al. (2017) reported a significant increase in Chinese cabbage, which yielded 80% more under fish effluent irrigation than conventional production. It is of great benefit at both community and family levels that such integrated aquaculture and agriculture systems are critically important to achieve on-farm sustainability and global food security. Indeed as freshwater demand increases, global water scarcity is gradually increasing at an alarming rate. Therefore, increasing crop production with the least amount of water is of paramount importance to global food production. Integrated fish and rice farming have proved viable in so many Asian countries such as Bangladesh, India, Indonesia, Malaysia, and Vietnam, which has significantly improved both the social and economic livelihoods of people in those areas (Dey and Prein, 2004; Halwart et al., 2004; Suloma and Ogata, 2006). Similarly,

suppose these systems and methodologies are designed to suit the local environment and indigenous practices in similar countries like Egypt. In that case, they will create a sustainable and robust food production system that will enable so many families to shift out of poverty.

### 3.3. Effect of irrigation treatments on essential oil yield

The plants' essential oil content and yield at the cutting stage were assessed under different treatments for both cut one and two (Figure 4). Results of cut one show that FT and MT significantly had a higher oil content (0.69% and 0.63%) by fresh weight (100 g) compared to CT ( $p < 0.0001$ ). However, no significant difference in oil content was noted among all treatments at the second cut. Results of this study are also supported by Verma et al. (2010), who reported that the oil content of *Origanum* was highest at full bloom. Overall, plants in cut two had a higher oil content (0.78%) than in cut one (0.58%) and this was significantly different  $t(28) = -4.66, p < 0.0001$ . These results were higher compared to the 0.42% average essential oil content reported by Nurzyńska-Wierdak (2009) in his field trials on oregano plants.

At a larger scale comparison, FT can yield up to 64.02 and 143.5 dm<sup>3</sup> ha<sup>-1</sup> of essential oil for the first and second cuts, respectively. CT can yield only 15.95 and 109.33 dm<sup>3</sup> ha<sup>-1</sup> for the first and second cut, respectively, while MT can still give the yield between FT and CT, i.e., 38.17 and 117.69 dm<sup>3</sup> ha<sup>-1</sup> for the first and second cut, respectively. Considering the second cut results, this implies that FT can produce about 34.17 dm<sup>3</sup> ha<sup>-1</sup> of essential oil more than CT per cut. The cumulative yield can reach up to 103 dm<sup>3</sup> ha<sup>-1</sup> of essential oil yield difference between FT and CT in three cuts per year. Different studies have shown that nitrogen's application rates greatly alter essential oil content and composition (Ahl et al., 2009; Gürbüz et al., 2009b; Khater et al., 2015). The availability of high nutrient nitrogen in the rhizosphere enhances the chemical synthesis and composition of essential oils (Sharma & Kumar, 2012; Zheljzakov et al., 2010). These results agree with those of (Aziz et al., 2008; Omer, 1999), who found a positive correlation between nitrogen fertilization and essential oil content

Table 3. The chemical composition of *O. syriacum* at the harvesting stage for all treatments.

Component name	First cut			Second cut		
	CT	FT	MT	CT	FT	MT
α-Pinene	0.98 <sup>a</sup> ± 0.05	0.84 <sup>a</sup> ± 0.06	0.99 <sup>a</sup> ± 0.53	0.69 <sup>a</sup> ± 0.02	0.75 <sup>a</sup> ± 0.18	0.66 <sup>a</sup> ± 0.08
β-Pinene	0.39 <sup>a</sup> ± 0.06	0.39 <sup>a</sup> ± 0.04	0.29 <sup>a</sup> ± 0.04	0.18 <sup>a</sup> ± 0.04	0.21 <sup>a</sup> ± 0.07	0.17 <sup>a</sup> ± 0.03
β-Myrcene	1.37 <sup>a</sup> ± 0.33	1.31 <sup>a</sup> ± 0.31	0.60 <sup>a</sup> ± 0.42	1.84 <sup>a</sup> ± 0.14	2.14 <sup>a</sup> ± 0.45	1.95 <sup>a</sup> ± 0.19
α-Phellandrene	0.46 <sup>a</sup> ± 0.08	0.39 <sup>a</sup> ± 0.07	0.33 <sup>a</sup> ± 0.00	0.48 <sup>a</sup> ± 0.16	0.56 <sup>a</sup> ± 0.24	0.59 <sup>a</sup> ± 0.22
α-Terpinene	1.77 <sup>a</sup> ± 0.05	1.59 <sup>a</sup> ± 0.09	1.05 <sup>a</sup> ± 0.37	1.98 <sup>a</sup> ± 0.12	2.22 <sup>a</sup> ± 0.36	2.05 <sup>a</sup> ± 0.12
p-Cymene	10.75 <sup>a*</sup> ± 0.04	9.28 <sup>a*</sup> ± 1.60	9.15 <sup>a*</sup> ± 3.44	6.18 <sup>a*</sup> ± 0.47	6.38 <sup>a*</sup> ± 0.73	5.36 <sup>a*</sup> ± 0.50
γ-Terpinene	5.06 <sup>a*</sup> ± 0.58	4.81 <sup>a*</sup> ± 0.62	4.13 <sup>a*</sup> ± 0.99	7.28 <sup>a*</sup> ± 0.11	8.50 <sup>a*</sup> ± 0.85	7.27 <sup>a*</sup> ± 0.60
Terpinen-4-ol	0.65 <sup>a</sup> ± 0.05	0.50 <sup>a</sup> ± 0.00	-	0.32 <sup>a</sup> ± 0.15	0.71 <sup>a</sup> ± 0.40	0.50 <sup>a</sup> ± 0.13
Carvacrol	69.51 <sup>a*</sup> ± 2.83	80.87 <sup>a*</sup> ± 11.35	78.55 <sup>a*</sup> ± 1.82	73.71 <sup>a*</sup> ± 0.27	71.02 <sup>a*</sup> ± 4.04	74.70 <sup>a*</sup> ± 1.86
Caryophyllene	1.96 <sup>a</sup> ± 0.22	2.90 <sup>a</sup> ± 0.03	2.04 <sup>a</sup> ± 1.22	2.44 <sup>a</sup> ± 1.29	2.73 <sup>a</sup> ± 0.54	2.714 <sup>a</sup> ± 0.60
Caryophyllene oxide	0.46 <sup>a</sup> ± 0.02	0.33 <sup>a</sup> ± 0.00	0.31 <sup>a</sup> ± 0.00	0.52 <sup>a</sup> ± 0.12	0.48 <sup>a</sup> ± 0.13	0.37 <sup>a</sup> ± 0.11
Humulene	2.04 <sup>a</sup> ± 0.00	-	0.31 <sup>a</sup> ± 0.00	0.19 <sup>a</sup> ± 0.04	0.17 <sup>a</sup> ± 0.04	0.21 <sup>a</sup> ± 0.15
β-Bisabolene	0.41 <sup>a</sup> ± 0.00	-	0.58 <sup>a</sup> ± 0.00	0.41 <sup>a</sup> ± 0.21	0.49 <sup>a</sup> ± 0.13	0.50 <sup>a</sup> ± 0.27
Bicyclo [3.1.0]hex-2-	2.06 <sup>a</sup> ± 0.07	1.90 <sup>a</sup> ± 0.18	1.55 <sup>a</sup> ± 0.49	1.51 <sup>a</sup> ± 0.05	1.66 <sup>a</sup> ± 0.35	1.48 <sup>a</sup> ± 0.18
1-Octen-3-ol	0.76 <sup>a</sup> ± 0.54	0.41 <sup>a</sup> ± 0.01	-	-	-	-
Cis-Sabinene Hydrate	1.11 <sup>a</sup> ± 0.24	0.93 <sup>a</sup> ± 0.00	1.08 <sup>a</sup> ± 0.35	-	-	-
trans-3-Caren-2-ol	1.13 <sup>a</sup> ± 0.00	0.69 <sup>a</sup> ± 0.00	0.77 <sup>a</sup> ± 0.00	-	-	-
Thymoquinone	1.13 <sup>a</sup> ± 0.00	0.69 <sup>a</sup> ± 0.00	0.77 <sup>a</sup> ± 0.00	-	-	-
D-Limonene	-	-	-	0.45 <sup>a</sup> ± 0.03	0.45 <sup>a</sup> ± 0.12	0.48 <sup>a</sup> ± 0.04
Phenol, 2-methyl-5-(1-methylethyl)-, acetate	-	-	-	0.65 <sup>a</sup> ± 1.09	0.17 <sup>a</sup> ± 0.10	0.22 <sup>a</sup> ± 0.11
tau.-cadinol	-	-	-	0.23 <sup>a</sup> ± 0.03	0.26 <sup>a</sup> ± 0.08	0.23 <sup>a</sup> ± 0.01

Percentage Composition values are given as means of the three replicates for each treatment for the two cuts with their standard deviations. Values in the same row with the same letter within each cut are not significantly different ( $p > 0.05$ ). \* represents a significant difference of a component between the first cut and the second cut ( $p < 0.05$ ). Missing values for a component in specific treatments are denoted with -.

for *Origanum syriacum* and *Ocimum americanum*. Chauhan et al. (2013), in their field experiment with *Origanum vulgare* recorded a maximum oil content of 0.55% by fresh weight of 300 g. This study revealed more than 50% of the oil content that has been previously reported at full bloom.

### 3.4. Effect of irrigation treatments on essential oil composition

Results in Table 3 show the percentage composition of the 21 chemical constituents analyzed as an average of five samples for each treatment for both the first and the second cut. Among all the analyzed components, carvacrol was the most abundant, reaching 80.87% and 74.69% for the first and second cut. It was closely followed by *p*-Cymene ranging (5.364–10.75%),  $\gamma$ -Terpinene (4.13–8.498%), Caryophyllene (1.96–2.9%),  $\alpha$ -Terpinene (1.05–2.22%), and  $\beta$ -Myrcene (0.6–2.14%) in both cuts. The study results agree with the findings of (Ciamician, 2000; El-Wahab et al., 2016; Kokkini et al., 2003; O'Grady and Kerry, 2009) who found the same main constituents in *Origanum* crops. Carvacrol was highest, with FT reaching 80.87% for the first cut, but it dropped to 71% in the second cut. This might have been caused by the exceedingly cumulative levels of nutrient nitrogen in the root zone, as reported by (Azizi et al., 2009). Compared to all treatments, even though carvacrol, *p*-Cymene, and  $\gamma$ -Terpinene had quite different composition values, they were not found significantly different between treatments for the same cut number ( $p > 0.05$ ). Significant differences for the composition values were sighted only between the two cuts for the treatments ( $p < 0.0001$ ). This study results conform with Azizi et al. (2009), who experimented with different oregano varieties and reported more than 70% carvacrol as the main component in oregano closely followed by  $\gamma$ -terpinene and *p*-cymene at 8.1–9.5% and 4.5–5.3%, respectively. Also, in their previous experiment with *Origanum vulgare* (Chauhan et al., 2013), found up to 62.26% of thymol as the main chemical composition that is chemically a very close active ingredient to carvacrol. *p*-Cymene as the second major constituent was relatively higher up to 10.75%.

## 4. Conclusion

Findings showed that the herbage yield of *Origanum syriacum* L. could be significantly increased by irrigation with fish effluents (FT). Similarly, FT showed a greater potential of increasing essential oil yield per cut per area of production. However, it did not significantly increase the essential oil composition of the main compounds compared to the rest of the treatments. Overall, irrigation with FT, increases crop biomass yield and essential oil content of *O. syriacum*. These findings serve as a milestone towards fertilizer savings and water consumption regarding water resource sustainability and efficient use. With the additional crop (fish), FT can significantly increase the net benefits, water use efficiency, and better resource sustainability. A holistic scientific understanding of the synergies at all levels between the aquaculture and agricultural units is required to achieve these goals. This study can serve as a useful reference for case studies focusing on increasing on-farm productivity and sustainability. However, the most appropriate integrated systems should be country- and site-specific, considering the available local tools and materials.

Nevertheless, more studies are required to investigate further FT's effect on the essential oil composition over several cuts and in different seasons. Repetitive water analysis should be done to evaluate and assess the plants' entire growth cycle's water quality impact. We also suggest future research on the IAA system's economics, investigating the study's economic feasibility on different high-value crops and the water footprint of the system. It considers water recycling and re-uses, hence assessing its sustainability.

## Declarations

### Author contribution statement

Fahad Kimera: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Hani Sewilam: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Walid M. Fouad; Ashraf Suloma: Conceived and designed the experiments; Wrote the paper.

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### Data availability statement

Data included in article/supplementary material/referenced in article.

### Declaration of interests statement

The authors declare no conflict of interest.

### Additional information

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