

# Maximizing Phytochemical Content for Some Medicinal Plants along with Nile tilapia (*Oreochromis niloticus*) Yield under Two Different RAS Aquaponic Models

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## Abstract

Aquaponics is a promising system for producing high-quality medicinal plants with more pharmacological content along with enhanced fish yield. The aim of the study was to examine two recirculating aquaculture system (RAS) hydroponic models using two medicinal plants (*Salvia officinalis* and *Origanum majorana*) plant characters parallel with Nile Tilapia (*Oreochromis niloticus*) performance. Results revealed that using nitrate as nitrogen source (model B) showed superiority over model A (using ammonia as nitrogen source) in all plant characters in both tested plants under study. Gas Chromatography (GC) results showed a huge number of phytochemicals variety from monoterpene, sesquiterpene, diterpene, phenolic diterpene, fatty acid alkyne, fatty acid, Alkaloid and steroid. Also, Nile tilapia production indicators were significantly better in model B compared to model A. Furthermore, the average net yield and gross yield were increased. Gross yield values ranged from 6.72 to 8.30 kg/m<sup>3</sup> over 63 days. One of the major factors that could enhance fish performance in this study was the bioactive compounds that may be released into the water due to the cultivated plants. GC-Mass analysis indicated that both plants contained antioxidant compounds. Regarding plant type, it was obvious that the production was slightly higher in common sage treatments than in marjoram regardless of the models. Meanwhile, fish differently performed according to assorted models, where production indicators were better in model B (nitrate) than in model A (ammonia).

**Keywords:** Sage, Marjoram, Nile Tilapia, GS-MS, ammonia, nitrate, aquaponic.

## 1. Introduction

Aquaponic is a cutting-edge high lighting technology helping crops to adapt to climate change challenges (Aslanidou *et al.*, 2023). Unlike conventional agricultural resource management, an aquaponic model saves water and reduces waste, costs, and helps enhancing environmental contamination (Ibrahim *et al.*, 2023). Aquaponics, a mix of aquaculture and hydroponics, offers a sustainable and environmentally sound way to crop growing, particularly for medicinal plants. The fish tank's nutrient-rich water serves as a natural fertilizer for the plants, while the plants help filter the water, establishing a symbiotic connection to satisfy the United Nations' planned sustainable development objectives (Ibrahim *et al.* 2023)). Researchers have investigated the physiological and chemical changes noticed in medicinal plants growing at RAS aquaponic systems, with encouraging results (Dadras *et al.*, 2023). Researchers have investigated chemical changes at medicinal plants grown under RAS aquaponic systems, revealing promising outcomes (Liao *et al.*, 2022). RAS aquaponic systems help medicinal plants to enhance aquaponics to produce high-quality medicinal plants with enhanced nutritional content and

pharmacological activity (Patloková and Pokluda 2024). Aquaponic systems can be achieved by using medicinal plants with a consistent and ideal growing environment (Flores-Aguilar *et al.*, 2023). The nutrient-rich water from the fish tank provides plants with a steady supply of important elements including nitrogen, phosphate, and potassium. The nutrient-rich water from the fish tank can raise secondary metabolites level in plants, which are plant chemicals that are not required for plant development which may have pharmacological effect (Roslan *et al.*, 2021 and Mielcarek *et al.*, 2024). Aquaponic systems can boost the concentrations of antioxidants, anti-inflammatory substances, and other useful phytochemicals in medicinal plants. These chemicals can provide a number of health advantages, including lowering risks of cancer, heart disease, and stroke (Cuevas-Cianca *et al.*, 2023). This can lead to increased plant growth and biomass production (Trimble, 2022). Aquaponic systems can also help to reduce stress in plants, thus the constant flow of water through the system helps to regulate plant temperature and humidity as well as protecting plants from pests and diseases (Akpenpuun *et al.*, 2023). Aquaponics has proved to be successful and efficient for small and big scale outputs of lettuce, tomatoes, and other green salads (Ibrahim *et al.*, 2023). However, not all plant species can

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live and flourish under aquaponic systems; therefore, it is necessary to identify plant species that can be grown and produced under these systems, since biomass production is a function of survival and growth (Carlos Valdez *et al.*, 2020).

Aquaponics provides a distinct benefit in semi-intensive systems with moderate input levels by increasing the overall efficiency of fish production, especially for species like the Nile tilapia (*Oreochromis niloticus*), a crucial species in aquaculture worldwide because of its high growth rates and adaptability. Studies have indicated that aquaponic systems may significantly increase tilapia productivity and improve production efficiency (Barbosa *et al.*, 2020) by maintaining optimal water quality parameters, which are crucial for fish health and growth. Aquaponics is a highly effective and profitable method in semi-intensive settings since it incorporates hydroponic plants into these systems, which not only helps to purify the water, but also generates an additive value (Krastanova *et al.* 2022).

The current study aimed to:-

- Examine the impact of two different aquaponic models on the growth and chemical compositions of two medicinal plants, common sage (*Salvia officinalis*) and marjoram (*Origanum majorana*).
- Evaluate the influence of the growing plants and models on the **performance of Nile tilapia** (*Oreochromis niloticus*) fish.
- Optimize the use of water and feed unit to achieve maximum efficiency.

## 2. Materials and Methods

The experimental design took place at El-Max Station for Applied Research, National Institute of Oceanography and Fisheries, Alexandria Branch, with collaboration of El-Sabaheya Horticulture Research station (SHRS), Horticulture Research institute (HRI), Agriculture Research Center (ARC), Egypt.

### 2.1. Plant Material

Plants under study were Common Sage (*Salvia officinalis*) and Marjoram seedlings (*Origanum majorana*) supplied by Elqanater Aromatic and Medicinal Plants Research Dept., HRI, ARC, at the age of 3 weeks. Control was planted in pots while plant seedlings were transferred to the hydroponics unit in the floating raft technique and planted at a density of 10 plants per tray. The hydroponic

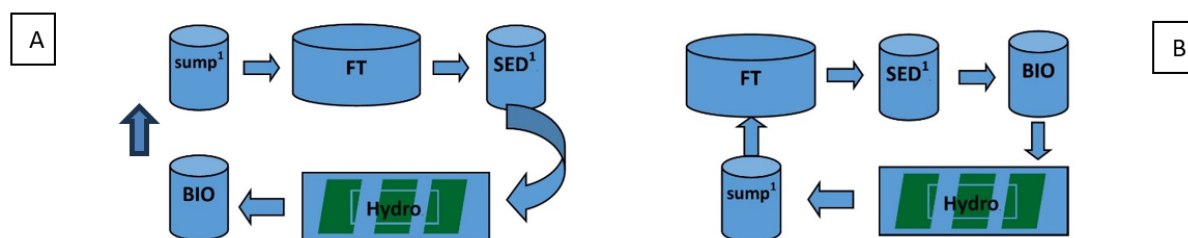
system was supplied with two different nitrogen sources, model A (before the biological filter): Ammonia and model B (after the biological filter): Nitrate. Plant vegetative characters were recorded. They include shoot and root length (cm), number of leaves, number of branches, fresh and dry weight for shoot and root (g).

### 2.2. Fish Material

Monosex Nile Tilapia (*Oreochromis niloticus*) fingerlings were obtained from a commercial fish hatchery in Kafr Elshikh, Egypt, and were transported to the experimental site through the aeration plastic tanks. The fish were stocked for acclimatization to the experimental conditions for 15 days in circular 300-liter freshwater tanks with continuous aeration, then transported to the aquaponics unit. Acclimatization period took 14 days in fresh water; about 30% of the water volume was regularly exchanged to remove the faecal matter and leftover feed. After acclimatization in the aquaponic system, Nile tilapia fingerlings were kept in 300-liter rearing tanks at a total biomass of about 1 kg/L. The mean weight of stocked fish was 28.22 g. It was given a commercial diet, to satiation, in the morning (8 am and 2 pm) that included 25% protein and 4% crude fat. Fish weight and other growth evaluation metrics were measured every 15 days using a digital balance (Generic, SF- 400A).

Four aquaponic units were investigated in this study; each consists of three circular fibreglass fish tanks of 500 L capacity (water volume 300 L) and three hydroponic plants grow beds of 0.52 m<sup>2</sup> (0.93 m × 0.56 m) area, fixed on a wooden table (Figure 1). The flood and drain system with gravity and pumps was used to regulate the water flow in the system sub-units. A submersible pump (SH-251, 25 W power) was used to pump water from the sediment /or biological filter to the hydroponic bed and from the sump to the fish tank. Throughout the experiment, a flow rate of 180 L hour<sup>-1</sup> was maintained, and the frequency of pumping was controlled by an automated timer (220v, time switch).

Continuous aeration was provided to each unit in the aquaponic system except the sediment tanks. The experiment followed a completely randomized design with four treatments allotted with different water flow orders as shown in Fig. (1) with two different plants. The same water unit was used along the whole experiment with no exchange rate except for a few amounts to compensate for normal evaporation losses.



**Figure 1.** Two sequences used in the aquaponic models according to water flow direction; Model A: (Ammonia): Fish tank (FT), Sediment tank (SED.), Hydroponic unit (Hydro), Biological filter (BIO.) and sump tank (sump) then to the fish tank again. Model B: (Nitrate): Fish tank (FT), Sediment tank (SED.), Biological filter (BIO.) Hydroponic unit (Hydro) and sump tank (Sump) then to the fish tank again. <sup>1</sup> submerged pump.

### 2.3. Essential oil extraction and GC-MS analysis:

Preparing plant samples for Gas Chromatography-Mass Spectrometry (GC-MS) involves sample collection and

preparation choose healthy plant material representative of the study. Thoroughly wash the plant material to remove any surface contaminants (soil, dust, etc.). Dry the material

to remove excess moisture. Reduce the plant material to a fine powder using a grinder, mortar and pestle, or liquid nitrogen which is very important increases the surface area for efficient extraction. Ethanol solvent was selected based on the polarity of the target compounds.

"Sage and Marjoram's chemical profiles were analyzed using GC-MS. Extracted oils were dried and then injected (1  $\mu$ L, split mode) into a Trace GC-ISQ system equipped with a TG-5MS column. Helium (1 mL/min) was the carrier gas. A temperature gradient (50°C to 300°C) was applied to separate compounds, with a 4-minute solvent delay. Mass spectra (m/z 50-650, 70 eV) were acquired and compared to the NIST 05 database for identification as recommended by Mohamed et al. (2020). Following extraction, oil was dried with anhydrous sodium sulfate; the same was prepared for GC-MS analysis (Abd El-Kareem et al., 2016 and Farouk et al., 2018).

#### 2.4. Water quality analysis

The water quality parameters: temperature, pH and dissolved oxygen (DO) were weekly analyzed in situ using a portable multi-parameter (Lovibond, SensoDirect 150, Germany). Ammonia was analyzed by colorimetry after sample fixation for 8 hours and then measured by spectrophotometer according to Grasshoff *et al.* (2009) at wavelengths 550.

#### 2.5. Plant growth characteristics:

The following parameters were undertaken at 63 days after planting for the two studied species of medical plants, common sage (*Salvia officinalis*) and marjoram (*Origanum majorana*): plant length (cm), root length (cm), number of leaves per plant, number of branches per plant, fresh and dry plant weight (g), shoot fresh and dry weight (g), and root fresh and dry weight (g).

#### 2.6. Fish production calculation:

Fish production indicators were calculated according to the following equations:

Net fish yield (NFY) = Total fish harvested (kg) – Total fish stocked (kg)

Net annualized Production (NAP) = NFY x 365/Pond surface area  $m^2$  x Growth period in days

Net Biomass Gain (NBG) = Final Biomass– Initial Biomass

Net Fish Production = Final Biomass–

Initial Biomass/Area of Pond×Time Period Gross Yield (GY) = Final Biomass/Area of Pond

Net Annual Production (NAP) = Final Biomass–Initial Biomass /Area of Pond

Biomass increase% = (final biomass-initial biomass/initial biomass)\*100

**Table 1.** Mean performance for common sage plants under two different hydroponic models:

Treat.	Plant length (cm)	Root length (cm)	No. of leaves/plant	No. of branches/plant	Plant fresh weight (g)	plant dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Control	23.83±2.57b	24.87±1.63a	54.00±4.44b	5.33±0.58b	15.33±3.31b	3.99±0.67b	12.28±2.29b	2.66±0.43b	3.05±1.43a	1.33±0.25a
Model (A)	23.99±2.36b	25.56±3.08a	55.58±1.69ab	6.67±0.33a	13.98±1.65b	5.30±1.60ab	11.10±1.55b	3.53±1.15ab	2.87±1.15a	1.76±0.45a
Model (B)	29.39±2.55a	29.31±3.88a	62.18±4.01a	7.32±0.34a	22.87±2.78a	6.31±0.40a	17.44±1.23a	4.73±0.25a	5.43±2.94a	1.58±0.21a

Means having letter in common do not significantly differ, using Duncan's multiple range test at  $p=0.05$  level of significance.

The data of Table (2) indicate that the numbers of the studied characters of marjoram plants were significantly

#### 2.7. System Purification Efficiency and Ammonia Removal Amount:

Purification Efficiency percentage (PE%) was calculated according to the following equation:

PE% = [(ammonia in the production tank-ammonia after biofilter)/ammonia in the production tank] \*100

Amount of TAN removed (VTR/ $m^3$ /day) =  $[(NH_4-N_{in}-NH_4-N_{out}) * Q] / V_{media}$

When:  $NH_4-N_{in}$  and out: the concentration of TAN in and out of the hydroponic system ( $g/m^3$ ),

TAN: total ammonia nitrogen, VTR: volume of TAN removed.

Q: flow rate ( $m^3$ /day),

$V_{media}$  is the volume of filter media ( $m^3$ ).

#### 2.8. Statistical analysis

The study was designed as a Randomized Complete Design (RCD) and statistically analyzed using Costat version (6.4). Statistical package SPSS 24 was used to analyze fish performance data to compare the means of different parameters. Two-way ANOVA tests were used, and Tuckey's test was applied to define the significant difference ( $p \leq 0.05$ ) between the treatments for different parameters.

### 3. Results

This study was conducted to assess the effects of two different aquaponic models, referred to as Model A and Model B, on the growth performance of two medicinal plants, common sage (*Salvia officinalis*) and marjoram (*Origanum majorana*). The study focused on a numerous of plant growth parameters, including plant length, root length, number of leaves per plant, number of branches per plant, fresh and dry plant weight, shoot fresh and dry weight, and root fresh and dry weight. Additionally, the study examined the productivity and overall performance of Nile tilapia (*Oreochromis niloticus*) reared within these two aquaponic systems.

he data presented in Table (1) show that the majority of the studied growth characteristics of the common sage plants were significantly influenced by the varying hydroponic models, with the exceptions of root length and root fresh and dry weight characters. Among the models, Model (B) was found to produce significantly higher mean values for plant length (cm), number of leaves per plant, plant fresh weight (g), plant dry weight (g), shoot fresh weight (g) and shoot dry weight (g).

affected by the different hydroponic models. These include plant length (cm), root length (cm), fresh plant weight (g)

and shoot fresh weight (g). Among the tested models; Model (B) appeared to be the most promising, as it is possessed the highest mean values for these characters followed by model (A), meanwhile the control treatment gave the lower means. Each of the following character;

number of branches per plant, plant dry weight (g), shoot dry weight (g) and root fresh and dry weight (g) characters were not significantly affect by the two hydroponic models examined.

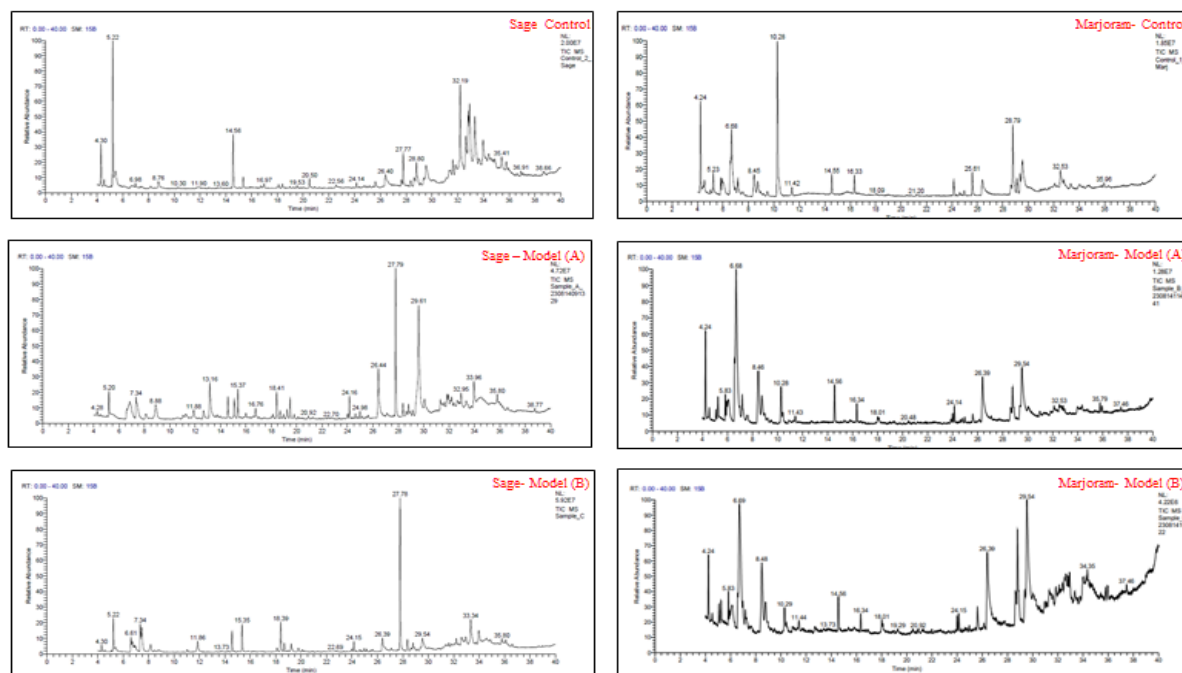
**Table 2.** Mean performance for marjoram plants under two different hydroponic models.

Treat.	Plant length (cm)	Root length (cm)	No. of Branches/plant	Plant fresh weight (g)	plant dry weight (g)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)
Control	20.76±1.85b	12.13±0.76c	10.07±1.81a	6.09±0.87b	3.00±0.63a	3.93±0.77b	2.17±0.48a	2.17±0.17a	0.83±0.17a
Model (A)	22.72±1.07ab	15.05±0.79b	12.89±1.35a	6.83±0.74b	3.87±0.51a	4.51±0.61b	2.77±0.34a	2.31±0.20a	1.09±0.18a
Model (B)	25.24±1.13a	17.58±1.04a	14.55±3.75a	9.19±0.48a	3.84±0.50a	6.71±0.19a	2.80±0.37a	2.49±0.47a	1.03±0.14a

Means having letter in common do not significantly differ, using Duncan's multiple range test at p= 0.05 level of significance.

Figure 2 shows GC-MS spectrum of common sage and marjoram with x- axis and y-axis showing the retention time (min) and the relative abundance area, respectively. Common sage (*S. officinalis*) GC-MS analysis results revealed 50 bioactive phytochemical compounds identified from different growing models (Table 3). Here, we will focus on the primary bioactive compounds that serve as the basis for cultivating Common sage and evaluate the extent to which their concentrations are influenced by various hydroponic and conventional agricultural systems. The key compounds of interest include Eucalyptol, Podocarpa-1,8,11,13-tetraen-3-one, 14-isopropyl-1,13-dimethoxy, and Carnosol. The findings presented in Table 3 indicate that the control treatment exhibited the highest concentrations of both eucalyptol and carnosol. These values were followed by those observed in hydroponic model B, whereas hydroponic model A displayed the lowest concentrations for these two compounds. Moreover, the control treatment and hydroponic model A produced comparable concentrations of the compound Podocarpa-1,8,11,13-tetraen-3-one, 14-isopropyl-1,13-dimethoxy. In contrast, this particular compound was completely absent in hydroponic model B.

Marjoram (*O. majorana*) GC-MS analysis results revealed 38 bioactive phytochemical compounds identified from different growing model designs (Table 3). Different filter arrangements of each model design revealed different metabolite as there were unique compounds for each, as well as common compounds among them as illustrated in fig (4). Marjoram cultivation primarily focuses on three active compounds: Sabinene, 4-Thujanol-CIS, and Z-Sabinene hydrate. Regarding the compound Sabinene, the data presented in Table (4) indicate that its concentration was highest under the control treatment, followed by the Model A system. The Model B system demonstrated the lowest concentration of this particular compound. The estimates for the 4-Thujanol, CIS compound, analyzed in three separate parts, showed variability without any clear trend in the data to assess the impact of the three treatments on the compound's concentration. Regarding the Z-Sabinene hydrate compound, the data presented in Table (4) indicated that its highest concentration was achieved under the control treatment, with a significant margin compared to hydroponic systems A and B.



**Figure 2.** GC-MS spectrum of common sage and marjoram.

**Table 3.** Chemical Composition (%) of sage and their treatments under study as inspected by (GC-MS).

Compound name	RT	Cas number	Library*	Terpene type	Control	Model A	Model B
a Pinene	4.3	127-91 -3	M	Mono	4.48	0.47	1.31
$\beta$ -Pinene	4.54	18172-6 7-3	R	Mono	0.84	0	0
Eucalyptol	5.22	470-82 -6	M	Mono	16.54	2.51	6.59
3-Isothujone	6.61	471-15 -8	R	Mono	0	0	2.42
3-Thujanol	6.84	513-23 -5	W	Mono	0	1.13	0
Camphore	7.34	464-48 -2	W	Mono	0	2.31	4.5
(+)-2-Bornanone	7.45	464-49 -3	R	Mono	0	2.41	5.64
endo-Borneol	8.16	507-70 -0	R	Mono	0	2.15	2.11
$\alpha$ -Terpineol	8.76	98-55-5	W	Mono	1.12	0	0
Estragol	8.88	140-67 -0	R	Mono	0	2.14	0
(1R)-(+)-pulegone	11.86	54345-6 1-8	M	Mono	0	1.21	2.36
trans-Isoeugenol	12.66	97-54-1	M	Mono	0	1.16	0
Methyl cis-cinnamate	13.15	103-26 -4	M	Mono	0	4.71	0
Caryophyllene	14.56	87-44-5	M	Sesqui	6.9	2.46	3.72
cis- $\alpha$ -Bergamotene	15.08	17699-0 5-7	R	Sesqui	0	2.36	0
a-Humulene	15.35	6753-9 8-6	C	Sesqui	1.39	3.27	4.9
6-epi- $\beta$ -Cubebene	16	13744-1 5-5	R	Sesqui	0	0.57	0
cis- $\gamma$ -cadinene	16.76	39029-4 1-9	R	Sesqui	0	1.13	0
Epiglobulol	18.39	552-02 -3	W	Sesqui	0	3.11	5.58
Humulene epoxide I	18.67	19888-3 4-7	M	Sesqui	0	0.87	1.57
Caryophyllene oxide	19.22	1139-3 0-6	W	Sesqui	0	1.39	1.96
$\tau$ -Cadinol	19.48	5937-1 1-1	R	Sesqui	0	2.38	0
Epiglobulo	20.5	NA	M	Sesqui	1.21	0	0
6,10,14-pentadecanone	24.01	502-69 -2	R	Sesqui	0	0.56	0
Ethanol, 2-(9-octadecenyloxy)-, (Z)-	24.14	5353-2 5-3	M	Fatty acid	0.66	0	0
Neophytadiene	24.15	504-96 -1	M	Diter	0	2.35	2.02
17-Octadecynoic acid	24.63	34450-1 8-5	M	Fatty acid alkyne	0	0.52	0

\*Library: R = Replib, W = WileyRegi, M = Mainlib, C= CaymanSp.

Table 3. Continued

Compound name	RT	Cas number	Library*	Terpene type	Control	Model A	Model B
cis-Phytol	24.98	102608-53-7	M	Diter	0	0.76	0
Hexadecanoic acid	26.39	57-10-3	W	Fatty acid	2.54	6.11	3.75
Epimanol; 1-Naphthalenepropanol	27.78	1438-6 2-6	R	Diter	4.17	17.26	28.14
Aromadendrene	28.36	489-39-4	W	Sesqui	0.88	1.6	2.16
Methyl 10-octadecenoate	28.8	13481-9 5-3	W	Fatty acid methyl ester.	3.96	1.4	1.51
Isohiapin B	29.13	NA	W	Sesquit	0	0.72	0
Oliec acid	29.54	03-Jul	R	Fatty acid	2.73	13.94	5.04
1H-Pyrido[3,4-B]indole-1-Bu Tanol $\zeta$ -Sec-Butyl-2,3,4,9-Tetrahy	31.32	14358-6 0-2	W	NA	0	1.36	0
3,4,7,8-Tetraazatricyclo[4.2.2.0(1,5)] dec-9-ene-3,4:7,8-bis(N-methylcarboximide), 2,2-diphenyl-	31.34	NA	W	NA	0	0	2.37
Cortisone Acetate	31.63	50-04-4	R	Steroid hormone	2.25	0	0
Podocarpa-1,8,11,13-tetraen-3-one, 14-isopropyl-1,13-dimethoxy	31.84	18326-2 0-0	W	Diter	1.55	1.45	0
Isoboldine	31.95	5140-28-3	C	Alkaloid	0	1.4	0
Naphtho[2,3-C]furan-1,3-Dio Ne, 6,7-Bis (Trimethylsilyl)-	32.61	80964-2 4-5	W	NA	4.8	2.48	0
Podocarpa-1,8,11,13-tetraen-3-one, 14-isopropyl-1,13-dimethoxy	32.64	18326-2 0-0	M	Diter	9.28	0.95	0
Pregnenolone	32.83	145-13-1	W	Steroid	6.07	0	0
Trimethylsilyl estrone	32.94	1839-5 4-9	R	NA	0	1.75	1.62
Carnosol	33.34	92519-8 2-9	W	Phenolic diterpene	7.18	1.66	2.17
Ferruginol	33.35	514-62-5	W	Diter	3.87	3.35	0
9-Antheracenol, 1,4,8-Trimethoxy-2-Methyl	33.95	70946-2 6-8	W	NA	0	3.27	3.57
17 $\alpha$ -Hydroxypregnenolone	35.41	387-79-1		Steroid	1.94	0	0
Benzenamine, 2-(6,7-Dimethoxy-2-Quinonlin)-4,5-Dimethoxy	35.79	76798-5 0-0	W	NA	1.32	0	1.72
1-Linolenylglycerol, 2TMS derivative	35.8	55521-2 2-7	W	NA	0	1.15	0
Total percentage of monoterpene					22.98	20.2	24.93
Total percentage of sesquiterpene					10.38	20.42	19.89
Total percentage of diterpene					31.12	27.32	31.62
Total percentage of phenolic diterpene					7.18	1.66	2.17
Total percentage of fatty acid alkyne					0	0.52	0
Total percentage of fatty acid					5.27	20.05	8.79
Total percentage of Alkaloid					0	1.4	0
Total percentage of steroid					1.94	0	0

\*Library: R = Replib, W = WileyRegi, M = Mainlib, C = CaymanSp

**Table 4.** Chemical Composition (%) of marjoram and their treatments under study as inspected by (GC-MS).

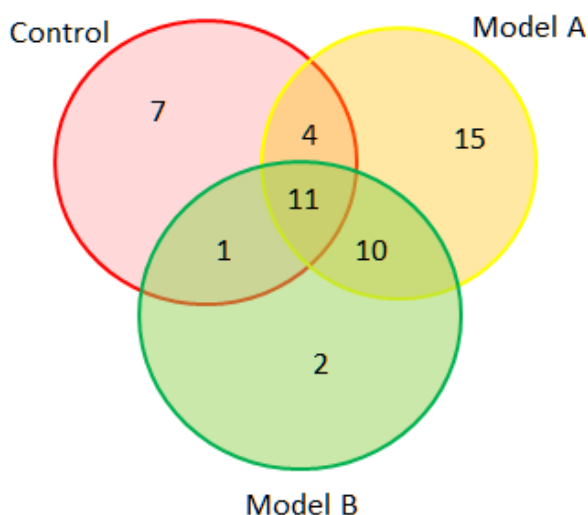
compound name	RT	Cas number	Library*	Terpene type	Control	Model A	Model B
(+)-Sabinene	4.24	3387-41-5	R	Mono	10.1	9.12	5.73
β-Pinene	4.54	18172-6 7-3	R	Mono	1.19	1.51	0
1,3,6-Octatriene, 3,7-dimethyl-	5.01	18172-67-3	W	Mono	2.56	0.57	1.06
α-Terpinolene	5.08	586-62-9	M	Mono	0	0.89	1.07
4-Thujanol,	5.23	17699-16-0	W	Mono	2.56	0	0
(+)-3-Carene	5.24	3387-41-5	W	Mono	0	2.72	2.13
ç-Terpinene	5.83	99-85-4	R	Mono	1.53	2.22	2.35
ç-Terpinene	5.93	99-85-4	R	Mono	3.05	1.59	0
Trans-4-Thujanol	6.04	15537-55-0	M	Mono	0	3.16	0
4-Thujanol, CIS-(+)-	6.56	15826-82-1	W	Mono	2.98	5.59	2.15
4-Thujanol, CIS-(+)-	6.68	15826-82-1	W	Mono	10.55	19.28	19.09
4-Thujanol, CIS-(+)-	6.98	513-23 -5	W	Mono	0.42	0	1.07
Trans-4-methoxy thujane	7.17	1100111-06-5	M	Mono	1.76	1.4	0
Terpinen-4-ol	8.44	562-74-3	M	Mono	4.09	7.6	7.7
L-à-Terpineol	8.74	98-55-5	W	Mono	2.11	2.89	0
Sabinene hydrate isomer	9.48	15537-55-0	R	Mono	0.77	0	0
Z- Sabinene hydrate	10.28	15537-55-1	R	Mono	21.6	9.88	5.62
(1R)-(+)-Pulegone	11.86	54345-6 1-8	M	Mono	1.31	0	0
Caryophyllene	14.56	87-44-5	M	sesqui	2.51	4.03	3.43
ç-Elemene	16.33	100762-46-7	W	Sesqui	2.32	2.62	3.34
Neophytadiene	24.15	504-96 -1	M	Diter	2	1.44	0
cis-Phytol	24.98	102608 -53-7	M	Diter	0.67	0	0
Palmetic acid	25.06	112-39-0	W	Fatty acid	2.99	1.25	2.34
Hexadecanoic acid	26.39	57-10-3	W	Fatty acid	2.38	1.25	6.1
Linoleic acid ethyl ester	28.62	544-35-4	R	Fatty acid	1.29	1.71	3.54
10-Octadecenoic acid, methyl ester	28.8	13481-9 5-3	W	Fatty acid methyl ester.	9.93	4.04	8.72
Methyl 10-octadecenoate	28.91	56554-49-5	M	Fatty acid methyl ester.	0	1.12	1.68
Isopropyl palmitate	29.36	56051-5	M	isopropyl alcohol	1.62	0.86	0
Oliec acid	29.54	03-Jul	R	fatty acid	3.53	4.25	5.99
Phenanthrene methanol	32.35	24035-43-6	M	Sesqui	3.53	4.25	0
Naphtho 3-Cjfurane-1,3-Dio Ne, 6,7-Bis (Trimethylsilyl)-	32.61	80964-2 4-5	W	NA	0	2.01	6.21
1-Phenanthrenemethanol, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-d imethyl-7-(1-methylethyl)-, [1S-(1à,4aà,10aá)]-	32.5	24035-43-6	M	NA	2.71	0	0

\*Library: (R = Replib, W = WileyRegi, M = Mainlib).

Table 4. Continued

Compound name	RT	Cas number	Library*	Terpene type	Control	Model A	Model B
Pregnenolone	32.83	145-13 -1	W	Steroid	0.64	0	0
Trimethylsilylestrone	32.94	1839-5 4-9	R	NA	0	0	3.25
Carnosol	33.34	92519-8 2-9	W	Phenolic diterpene	0	0	5.91
Benzenamine, 2-(6,7-Dimethoxy-2-Quinolin YL)-4,5-Dimethoxy	35.79	76798-5 0-0	W	NA	0	1.05	0
Behenic acid	35.96	929-77-1	W	Saturated fatty acid	0.86	0	0
Total percentage of monoterpene					66.58	68.42	47.97
Total percentage of sesquiterpene					8.36	10.9	6.77
Total percentage of diterpene					2.67	1.44	0
Total percentage of phenolic diterpene							
Total percentage of fatty acid					10.19	8.46	17.97
Total percentage of fatty acid methyl ester					9.93	5.16	10.4
Total percentage of isopropyl alcohol					1.62	0.86	0
Total percentage of steroid					0.64	0	0
Total percentage of Phenolic diterpene					0	0	5.91
Total percentage of Saturated fatty acid					0.86	0	0

\*Library: (R = Replib, W = WileyRegi, M = Mainlib).

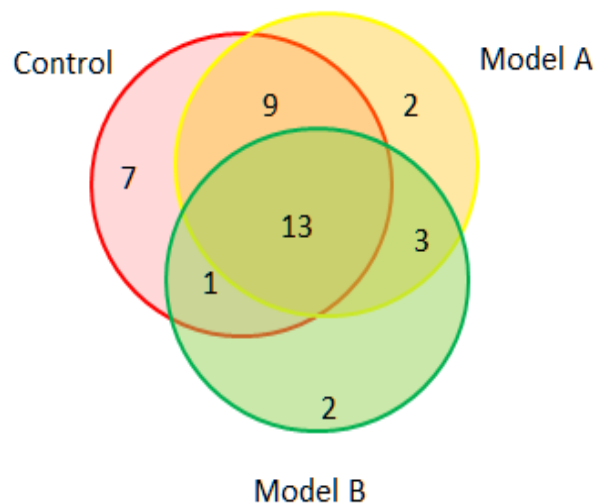


**Figure 3.** Common Sage GC-MS spectrographs for bioactive terpenoids.

### 3.1. Fish production parameters:

Table (5) presents the parameters related to fish production, including net biomass gain (NBG, g), net fish yield (NFY (g/m<sup>2</sup>), net annual production (NAP g/m<sup>2</sup>), Gross yield (g/m<sup>2</sup>), total biomass at harvest (g), total biomass at stocking (g) and biomass increment (%).

Figure (5) illustrates the main effect of the independent variable, represented by hydroponic models, on Nile tilapia production parameters. The data demonstrate that model B significantly gave the highest mean values across most studied fish production parameters. However, for the TBMs parameter, no significant difference was observed between models A and B (Fig. 5). Regarding the influence of the main effect of plant species on Nile tilapia production parameters, the findings of Figure (6) indicate

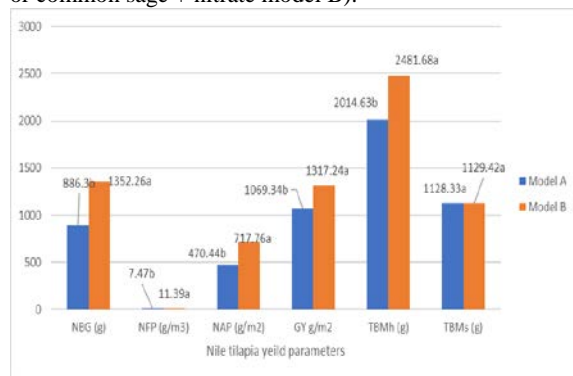


**Figure 4.** Marjoram GC-MS spectrographs for bioactive terpenoids

that no significant differences were noted between the effects of common sage and marjoram plants on all the studied production parameters of Nile tilapia.

Concerning the interaction between the Recirculating Aquaculture System RAS-Aquaponic Model and the cultivated plants, the data presented in Table (5) demonstrate that the MN treatment gave the highest mean values across all measured fish production parameters; NBG (1363.88 g), NFY (11.49 g/m<sup>2</sup>), NAP (723.93 g/m<sup>2</sup>), GY (1321.77 g/m<sup>2</sup>), TBM<sub>h</sub> (2490.22 g), TBMs (1126.33 g) and biomass increment (121.18 %) without significant differences with the treatment SN. Conversely, the two treatments of MA and SA possessed the lowest mean values in this respect. These findings suggest that the highest productivity of tilapia fish can be achieved under the conditions of this experiment by integrating these fish

into a hydroponic system B using marjoram or common sage as the cultivated plants (marjoram + nitrate model B or common sage + nitrate model B).



**Table 5.** Mean fish yield indicators under different RAS-Aquaponic Schemes for 63 days

Treatments	NBG (g)	NFY (g/m <sup>2</sup> )	NAP (g/m <sup>2</sup> )	GY (g/m <sup>2</sup> )	TBM <sub>h</sub> (g)	TBM <sub>s</sub> (g)	Biomass increase (%)
Common sage + Ammonia (Mod. A) (SA)	912.00 ± 48.87b	7.68 ± .41b	484.08 ± 25.94b	1082.09 ± 30.34b	2038.67 ± 57.16b	1126.67 ± 10.35b	80.9 ± 4.623b
Common sage + Nitrate (Mod. B) (SN)	1340.63 ± 60.95a	11.30 ± .51a	711.59 ± 32.35a	1312.70 ± 29.92a	2473.13 ± 56.38a	1132.50 ± 12.22a	118.46 ± 7.32a
Marjoram + Ammonia (Mod. A) (MA)	860.60 ± 112.14b	7.25 ± .94b	456.80 ± 59.52b	1056.58 ± 61.52b	1990.60 ± 115.91b	1130.00 ± 11.72b	76.12 ± 11.75b
Marjoram + Nitrate (Mod. B) (MN)	1363.88 ± 22.56a	11.49 ± .19a	723.93 ± 11.98a	1321.77 ± 4.30a	2490.22 ± 8.090a	1126.33 ± 14.90a	121.18 ± 4.32a

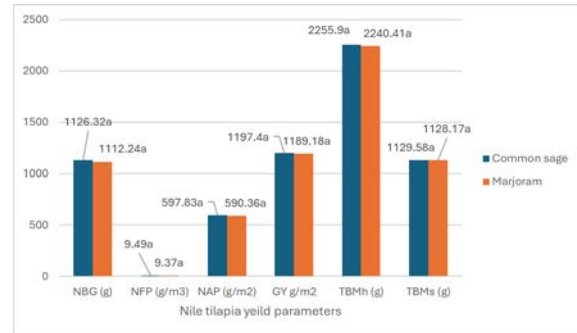
NBG: net biomass gain, NFY: net fish yield, NAP: Net Annualized Production, GY: Gross yield, TBM<sub>h</sub>: total fish biomass at harvest and TBM<sub>s</sub>: total fish biomass at stocking

### 3.2. System Purification Efficiency and Ammonia Removal Amount:

The purification efficiency of the different models during the experimental period was presented in fig. (7). The statistical analysis showed significant differences between models A and B (10.94 and 30.32%, respectively). When model B attained better system purification efficiency, however, the plant species did not show any significant differences in PE%. Marjoram models achieved better PE% (14.10% and 31.99% in models A and B, respectively). Nonetheless, common sage models performed only 7.78% and 28.64% in models A and B, respectively (fig. 8).

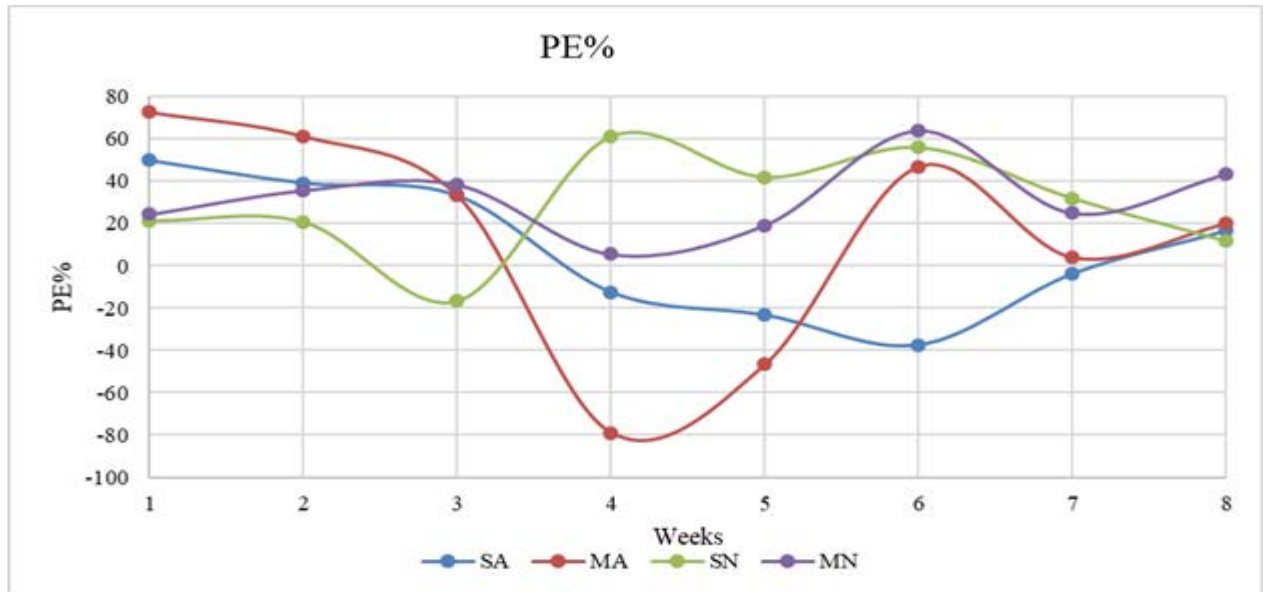
Concerning the TAN removal rate, fig. (9) illustrates the mean values of VTR (volume of TAN removed mg/m<sup>3</sup>/day) during the experimental period. The fourth week is considered the best in terms of VTR values, while the first week is the lowest, with significant statistical differences observed between the two weeks. For the remaining weeks, there was consistency in ammonia levels across the secondary units. As a result, the VTR rate was often negative, which can be recognized to the reduction of ammonia level in both the sump and the biological filter.

**Figure 5.** Nile tilapia yield parameters according to models (A and B)

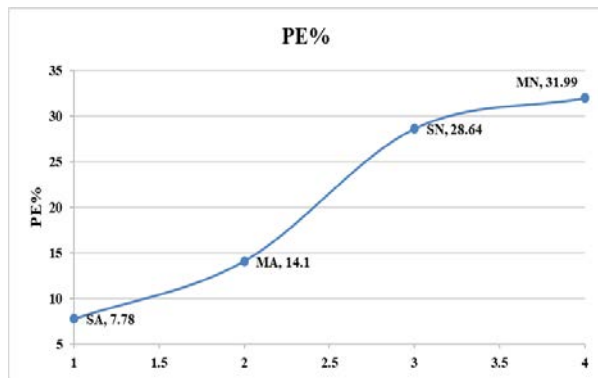


**Figure 6.** Nile tilapia yield parameters according to plant species (common sage and marjoram)

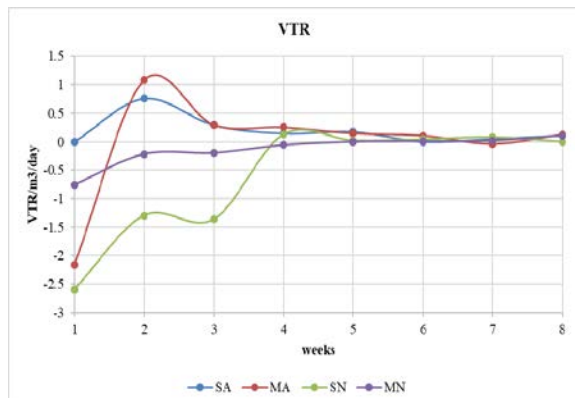
In biological systems like biofilters, nitrifying bacteria convert ammonia to nitrates through nitrification processes. By the fourth week, bacterial activity may have increased due to stabilized environmental conditions (such as temperature, oxygen levels, and pH), leading to a reduction in ammonia concentration. Over time, the biofilter may become more effective at removing ammonia as a thicker, more active bacterial biofilm forms on its surface, enhancing the biological oxidation of ammonia. Mostly, the best VTR values were obtained by marjoram compared to common sage. For Model A, the highest value was recorded in the second week (1.08), achieved by marjoram. Meanwhile, the best value obtained by common sage was (0.7560). Considering Model B, the best VTR value recorded by common sage was 0.0779 in the seventh week, while the highest VTR rate recorded by the marjoram was 0.1072, in the eighth week, followed by the seventh week (0.0195). Therefore, the weeks with the best VTR values recorded for sage were the first, fourth, fifth, and seventh, while for marjoram the best values were recorded in the second, third, sixth, and eighth weeks in both tested models.



**Figure 7.** Purification efficiency (PE %) values for the different models during the experimental weeks. Model abbreviations: SA (Sage Model A), MA (Marjoram Model A), SN (Sage Model B), MN (Marjoram Model B).



**Figure 8.** Mean purification efficiency (%) during the experimental period. Model abbreviations: SA (Sage Model A), MA (Marjoram Model A), SN (Sage Model B), MN (Marjoram Model B).



**Figure 9.** Mean volume of total TAN removed (VTR) values for the two models during the experimental period. Model abbreviations: SA (Sage Model A), MA (Marjoram Model A), SN (Sage Model B), MN (Marjoram Model B).

**Water quality:**

Water physicochemical parameters are presented in Table (6). The data revealed that there were no significant differences in temperature, pH and NH4 values between the different treatments. Nonetheless, DO attained significant differences when DO values were better in model A than in model B, in the hydroponic sub-unit.

Table: (6). Mean values of physicochemical water parameters during the experimental period

Parameter	Unit	SA (model A)	SN (model B)	MA (model A)	MN (model B)
Temp.	FT	26.29	26.33	26.35	26.63
	HP	26.85	27.16	27.08	27.46
pH	FT	7.92	7.93	7.76	7.78
	HP	7.79	7.98	7.79	7.85
DO	FT	5.93	5.72	5.87	5.95
	HP	6.06a	5.89ab	6.02a	5.75b
NH4	FT	0.12	0.22	0.14	0.12
	HP	0.09	0.17	0.1	0.08

SA (Sage Model A), MA (Marjoram Model A), SN (Sage Model B), MN (Marjoram Model B).

FT: fish tank, HP: hydroponic

**4. Discussion**

Overall, Plants grown under the nitrate model (B) showed higher performance concerning the studied characters. One of the key factors influencing plant growth in hydroponic models is the nitrogen source. Nitrate and ammonium are two primary forms of nitrogen that can be used in hydroponics. While both are essential for plant growth, there are distinct advantages and disadvantages in using each (Hilty *et al*, 2021). Many plants prefer nitrate as their major nitrogen supply because it may be immediately taken from the solution, which reduces the plant's metabolic burden. Nitrate absorption raises the pH of the

nutrient solution, which helps most plants maintain a healthy pH range. It is also less hazardous to plants than ammonium, particularly at higher doses. Ammonium absorption can reduce the pH of the nutrient solution, potentially causing root damage and nutritional shortages if not controlled effectively (Shilpha *et al.*, 2023). Plants must convert ammonium to nitrate before it can be used. This procedure consumes energy and may delay plant development. Also, high ammonium concentrations can be toxic to plants (Norton and Ouyang, 2019).

Research and applications of medicinal plants are increasing every day due to the beneficial phytochemicals that can enhance the development of new medicines. Phytochemicals are naturally produced biologically active chemical compounds that occur in various parts of plants that promote human hygiene and avoid against diseases. Today, about 80% of the population in the developing world uses phytochemicals as traditional medicines for health care. Most of these phytochemicals are also found in the Lamiaceae family specially in common sage (*S. officinalis*) as described by (Ali *et al.*, 2023) and marjoram (*O. majorana*). In fact, the various biological properties and various disease-preventive potential of *S. officinalis* and *O. majorana* are supposed to be primarily due to the presence of these kinds and concentrations of phytochemicals. Additionally, there are a promising link between the different aquaponic models and the growth and chemical content. These previous results referee to the effect of various aquaponic models on the growth and chemical content of *S. officinalis* and *O. majorana*. And these results were in line with Corrêa and Navarro (2024); Knaus *et al.* (2022); Knaus *et al.*, (2020); Hundley *et al.*, (2018); Hundley *et al.* (2013); they studied the effect of various Aquaponic models on the growth and chemical content of some medicinal plant such as; *O. majorana* and *O. basilicum*.

Nile tilapia perform differently according to assorted models, where production indicators were better in model B than in model A. Furthermore, the average net yield and gross yield were above averages in previous work. Akter *et al.* (2023) illustrated that the fish yield ranged from 4.60 to 5.47 kg/m<sup>3</sup>/65 days of culture at the end of the trial. Earlier studies revealed that, tilapia production in aquaponics systems was reported to be 9.59 kg/ m<sup>3</sup>/160 days by Jahan (2014) and 13.43 kg/m<sup>3</sup>/180 days by Bethe *et al.* (2017). In this present study, the gross yield ranged from 6.72 to 8.30 kg/m<sup>3</sup> within 63 days. This relatively high yield may be due to the semi-intensive stocking density which enables the fish to move and obtain the food. Likewise, the high quality of water positively affected the Nile tilapia's performance. One of the major factors that may enhance the fish performance in this study is the bioactive compounds that may be released into the water due to the cultivated plants, when GC-Mass analysis indicated that both plants contain antioxidant compounds. These compounds can leach into the water column, either through root exudations or decomposition of plant matter, and directly or indirectly benefit fish health. For instance, the antioxidant properties of these compounds can mitigate oxidative stress in fish, which is often induced by poor water quality or high stocking densities. When Oxidative stress can impair growth, immune function, and overall survival, the presence of antioxidants in the water can neutralize reactive oxygen species (ROS) and enhance fish

resistance (Rashidian *et al.*, 2021). Several studies have highlighted the positive effects of medicinal plants on aquatic organisms. For example, the inclusion of oregano leaf extracts (a close relative of marjoram) in fish diets has been shown to improve growth rates, feed conversion ratios, and immune responses in Nile tilapia (El-Bab *et al.*, 2024). Similarly, Metin *et al.* (2024) stated that sage extracts have been reported to enhance the antioxidant status and disease resistance of common carp (*Cyprinus carpio*). In the context of aquaponics, the leaching of these bioactive compounds into the water can provide similar benefits without the need for direct dietary supplementation.

When, Yang and Kim (2019) studied Nile tilapia biomass increase percentage, obtained only 27 %; however, in this current study, the biomass increase ranged from 76.12075 % to 121.18426 %. This elevated percentage is due to the size of stocked fish when the authors used adult Nile tilapia ( $\approx$  183 gm); nevertheless, the stocked fish were only  $\approx$  25gm. Lopez *et al.* (2013), studies supported the study explanation, and a growth increase was obtained ranging from 640.46 to 827.30% when they tested the performance of Nile tilapia dry weighed about 1.5 g.

When concerning plants used, it was obvious that the production was slightly higher in common sage treatments compared to those with marjoram treatments regardless of the applied models.

PE% in the current study was relatively lower than in other studies (Hamid *et al.*, 2022), they revealed that, PE% ranged from 92.49% to 61.26%. The lower PE% in the current study was due to the low TAN concentration all over the systems. Furthermore, the nature of media in biological filters plays a critical role in PE%, as the high porosity of the media enhances PE%.

Additionally, TVR values illustrated that, marjoram roots had better efficiency in absorbing ammonia from water than common sage roots ability. Moreover, PE% varied according to the flow rate and stocking density of fish when the flow rate was inversely proportional to PE% of the system (Wambua *et al.*, 2021).

## 5. Conclusion

Study objectives were testing the possibility of utilizing water from aquaponic (fish rearing) in producing medicinal plants with high economic value while maximizing the benefit from the water unit. RAS aquaponic systems offer a promising approach in cultivating medicinal plants with enhanced phytochemical content. The nutrient-rich environment and stable growth conditions foster increased biomass production and elevated levels of secondary metabolites. Further research is warranted to fully elucidate the potential of aquaponics in optimizing the production of high-quality medicinal plants while maximizing the benefit from the water (1 m<sup>3</sup>) unit. Additionally, the interaction between the aquaponic model and the type of plant cultivated played a significant role in the observed results. Model B consistently outperformed Model A across all fish production indicators. Likewise, the results provide a sign of the importance of selecting the appropriate aquaponic model and plant species to optimize water purification and total

ammonia nitrogen (TAN) removal, leading to better system stability and improved fish health and growth.

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### Ethical statement:

This proposal was reviewed and approved by the Institutional Animal Care and Use Committee of National Institute of Oceanography and Fisheries (NIOF-IACUC) under the approval code: NIOF-AQ1-F-22-R-032

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