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Influence of culture media and fulvic acid on *Thymus serpyllum* L. plant under hydroponic conditions

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Abstract
Thymus serpyllum (creeping thyme) is a newly introduced medicinal and aromatic plant in Egypt. It was grown hydroponically for this study. The goal was to assess the effect of three sandponic media (sand, silica, and sand with silica) and foliar spraying with fulvic acid (without and with) on plant growth, herbage yield, and essential oil yield. The experiment was designed as a split plot. The main plots involved fulvic acid spraying, while the subplots included various sandponic media. The results showed that cultivation in a sandponic system filled with an equal amount of silica and sand, combined with spraying plants with fulvic acid, produced the significantly highest fresh herb weight per square meter and the appropriate dry weight and essential oil yield. Control and other treatments, on the other hand, detected lower values. The dominant compound in essential oil was thymol. Depending on the treatments, it ranged from 41.80% to 53.45%.

Keywords: *Thymus serpyllum*, sandponic media, fulvic acid, essential oil

Introduction

Hydroponics is a method of growing plants in nutrient solutions, often with artificial mediums. Hydroponics technology has advanced. Efforts were made to conserve water and land while reducing labor costs to improve output and quality. The following are the primary benefits of hydroponics: water management, less water pollution, more efficient water and fertilizer use, a buffer capacity for mistakes, and lower costs are all benefits of this technology. The science of cultivation without soil uses inert materials such as sand, silica, vermiculite, and others. The hydraulic properties of the medium can be evaluated by considering the particle size and shape. The nutrition solution, with all the necessary elements, is added. The plant requires these components to meet its nutritional requirements for growth and development (Southern and King 2017) [1].

Sandponics is a hydroponic system for growing plants. It employs sand with low salt and mud content as a growing medium and a small portion of a low-dose nutrient solution to water the plants multiple times. Sandponic agriculture has the potential to grow crops for up to 25 years without the need to replace the substrate. It is a type of farming that is easy to manage, affordable, and open to those without experience in the field. Several studies have shown that particle size affects air permeability, water retention, and nutrient absorption in sandponics. Sandponics is an exciting new manufacturing method that is an ideal growing system for high-quality plant biomass (Brooke 2018; Wategreen 2020) [2, 3].

Egypt has recently had to increase the cultivation of new aromatic and medicinal plant species, primarily thyme, to keep up with demand on international markets for its exports of herbs and spices. Also, compared to varieties from other countries, the local *Thymus vulgaris* has a low yield and grows slowly.

Thymus serpyllum (creeping thyme) is a newly introduced plant. *Thymus serpyllum*, also known as Breckland thyme, is a Lamiaceae plant. It is a low-growing prostrate subshrub, a woody-stemmed creeping dwarf thyme native to Europe and Asia. It grows best on sandy hills, rocky outcrops, hills, banks, roadside sand, and sand banks. Its herb contains essential oil, the main constituent of which is thymol. It is possible to consume creeping thyme. It has a beautiful fragrance. As a result, it is common to include it in meals to enhance their flavor. The herb is used to make syrups, tinctures, infusions, decoctions, tea, and oil, among other natural herbal treatments.

It is an essential source of medicinal compounds with antioxidant, antibacterial, antitumor, and cytotoxic properties and their therapeutic applications. It is also used in the pharmaceutical, food, and cosmetics industries (Nikoli *et al*. 2014) [4].

Fulvic acid is a promising bio stimulant. It plays a vital role in the field of medicinal and aromatic plants. Fulvic acid is a humic substance that is a mixture of polyphenolic compounds formed by the breakdown of organic components. Some studies show that fulvic acid is more effective as a spray than humic acid. Fulvic acid has a lower molecular weight, more acidic groups, and a higher oxygen concentration than humic acid. Fulvic acid has excellent solubility in low pH conditions, which is typical of foliar sprays and contributes to its superior efficacy as a foliar spray compared to humic acid. Fulvic acid is significantly more effective as a foliar spray than a soil amendment. Fulvic acid, as a biostimulant, is a non-toxic water binder. It promotes plant productivity by increasing leaf uptake (Ali *et al*., 2022) [5].

Under hydroponically controlled conditions, we study the effects of three different sandponics soils, a foliar spray of fulvic acid, and the interactions between these factors on growth, production, and active constituents. This investigation aimed to get the most yield and quality from this newly introduced plant.

Materials and Methods

The experiment was done on a private farm in El-Obour City, Orabi Association (30° 13′, 59° N, 31° 32° E), Egypt, in 2021 and 2022, using a closed sandponics system inside a translucent polycarbonate greenhouse and 75% sunlight. The average greenhouse day and night temperatures were 28 °C and 23 °C, with a relative humidity of 65%. The study aimed to examine how three sandponics substrate materials, a foliar spray of fulvic acid, and their interactions affected the growth, production, and active constituents of *Thymus serpyllum*.

Concerning the sandponcis structure, three galvanized steel cultivation troughs with a height of 60 cm and a width of 2 m were built on the ground. With a 2% slope, the planting troughs were brought down to the solution recovery tank. As a waterproof layer, the inner wall of the trough was covered with 1,000 meters of plastic film. The cultivation trough's bottom section was v-shaped, and the drainage tubing was placed in the v-shape's center, serving as ventilation and nutrient solution recycling. The pipe body was separated by 20 cm of holes. The pipe body was covered with gauze to keep sand from clogging the drainage pipe hole. The troughs were rinsed with fresh water to remove excess salinity.

The seeds of *Thymus serpyllum* were imported from Gavrish Company, Moscow, Russia. Seeds were planted in the greenhouse on January 15th. Seedlings were cultivated in sandponics on March 15th. Plant-to-plant spacing was kept at 30 cm, and row-to-row distance was maintained at 30 cm. The trial density was nine plants per m^2 (Figure 1). The system was connected to a 10,000-liter tank containing a nutrient solution. The used nutrient solution had 1 mM $Ca(NO_3)_2$, 1 mM KNO_3, 1 mM $NH_4H_2PO_4$, 0.02 mM Fe-EDTA, 1 mM $MgSO_4$, 0.05 mM KCl, 0.025 mM H_3BO_3, 0.002 mM $ZnSO_4$, 0.0005 mM MoO_3, and 0.0005 mM $CuSO_4$ (Epstein 1972) [6]. All the recommended agricultural procedures were adhered to throughout the production stages.

The experiment was designed as a split plot. The main plots involved fulvic acid spraying, while the subplots included various sandponic media. The first trough of sandponics was filled with sand, the second with silica, and the third with a 1:1 mixture of the two types. Fulvic acid was sprayed on plants. It was prepared by dissolving 2.50 g of fulvic in 1 liter of water and sprinkled a month after transplanting and again after each cut. On the 15th of June and again on the 30th of September, two harvests of the blooming herb were carried out. It was cut 10 cm above the soil, with some branches remaining to regrow. ANOVA was used to analyze the data (Snedecor and Cochran 1982) [7], and LSD was used to test for 0.05 significance levels in mean differences.

The following information was recorded at the time of harvesting: growth and yield characteristics were represented by plant height in centimeters, fresh herb weight in grams per square meter, and dry herb weight in grams per square meter.

Quality parameters included the essential oil percentage in the air-dried herb by hydro distillation (British Pharmacopoeia 1963) [8]. The following equation was used to determine the essential oil yield per m^2 (ml): oil yield per m^2 = oil percentage x dry herb weight per m^2 /100. The oil was GC-MS analyzed using a Gas Chromatography-Mass Spectrometry instrument at the Laboratory of Medicinal and Aromatic Plants, National Research Center, Egypt, according to the specifications listed below. Device: a TRACE GC Ultra Gas Chromatograph (THERMO Scientific Corp., USA) coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system had a TR-5MS column (30 m x 0.32 mm i.d., 0.25 μm film thickness). Analyses were conducted using helium as carrier gas at a flow rate of 1.3 ml/min at a split ratio of 1:10 and the following temperature program: 80 ºC for 1 min; rising at 4ºC/min to 300ºC and held for 1min. The injector and detector were held at 220 and 200 ºC, respectively. Diluted samples (1:10 hexane, v/v) of 1 μL of the mixtures were continuously injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. The compounds were identified by comparing their retention times to those of authentic samples and by computer matching against commercial and library mass spectra constructed from pure substances (Massada 1976; Adams 2007; Babushok *et al*. 2011) [9-11].

Fig 1: *Thymus serpyllum* cultured in sandponics

Results and Discussion

I. Growth and yield characters

Tables (1-3) showed data on the effects of fulvic acid, cultivation media, and their interactions on growth and yield characteristics.

In terms of the impact of fulvic acid, the results showed that all fulvic acid-sprayed plants outperformed unsprayed plants. Foliage spray with fulvic acid significantly increased plant height, fresh herb weight per square meter, and dry herb weight per square meter over control. The first cut records were 44.58 cm, 486.58 g, and 165.04 g, while the second harvest records were 32.36 cm, 501.47 g, and 173.74 g.

In both cuts, the differences in plant height between the three cultivation media were insignificant. Furthermore, the sandponic system filled with sand and sand with silica produced a significantly higher maximum fresh herb weight per square meter than the other used materials. The first and second cut values for sand were 451.34 g and 462.27 g, while for sand with silica were 481.54 g and 495.29 g, respectively. Concerning dry herb weight per square meter, the maximum weights resulted from the system filled with silica and sand with silica with no significant variations. The first and second cut values for silica were 146.61 g and 153.01 g, while for sand with silica were 164.22 g and 168.71 g, respectively.

The responses of creeping thyme to the interactions between factors were as follows: in both cuts, plants grown in a blend of sand and silica and sprayed with fulvic acid significantly produced the highest fresh herb weight per square meter. These weights were 530.79 g and 548.11 g for the first and second cuts, respectively. With no discernible differences between them, cultivation in silica and sand with silica and spraying with fulvic acid revealed the heaviest dry weight per square meter. The first and second cut values for silica were 168.18 g and 170.90 g, while for sand with silica, they were 189.53 g and 191.09 g, respectively. On the other hand, plants grown in silica without fulvic acid spray had the poorest characteristics.

Table 1: Effect of fulvic acid, growth media, and their interaction on plant height (cm) (mean values of the two successive seasons).

Treatments	1st cut				2nd cut			
Fulvic acid	Growth media				Growth media			
	Sand	Silica	Sand + silica	Mean	Sand	Silica	Sand + silica	Mean
Without	41.50	38.00	39.08	39.53	25.25	21.00	23.08	23.11
With	44.58	42.17	47.00	44.58	27.50	37.33	32.25	32.36
Mean	43.04	40.09	43.04		26.38	29.17	27.67	
LSD 5%								
Fulvic acid	2.64				2.51			
Growth media	n.s.				n.s.			
Interaction	4.56				4.35			

Table 2: Effect of fulvic acid, growth media, and their interaction on fresh herb weight (g/m^2) (mean values of the two successive seasons).

Treatments	1st cut				2nd cut			
Fulvic acid	Growth media				Growth media			
	Sand	Silica	Sand + silica	Mean	Sand	Silica	Sand + silica	Mean
Without	435.29	353.35	432.29	406.98	446.83	359.13	442.46	416.14
With	467.40	461.56	530.79	486.58	477.70	478.60	548.11	501.47
Mean	451.34	407.46	481.54		462.27	418.87	495.29	
LSD 5%								
Fulvic acid	38.51				36.61			
Growth media	47.54				44.84			
Interaction	67.23				63.4			

Table 3: Effect of fulvic acid, growth media, and their interaction on dry herb weight (g/m^2) (mean values of the two successive seasons).

Treatments	1st cut				2nd cut			
Fulvic acid	Growth media				Growth media			
	Sand	Silica	Sand + silica	Mean	Sand	Silica	Sand + silica	Mean
Without	141.77	125.03	138.90	135.23	147.27	135.11	146.32	142.90
With	137.40	168.18	189.53	165.04	159.23	170.90	191.09	173.74
Mean	139.59	146.61	164.22		153.25	153.01	168.71	
LSD 5%								
Fulvic acid	19.45				17.06			
Growth media	23.81				n.s.			
Interaction	33.67				29.54			

II. Quality parameters

Tables (4-6) showed the impact of fulvic acid, cultivation media, and their interactions on essential oil attributes such as oil percentage and yield.

It was revealed that fulvic treatment considerably improved the aroma characteristics, which may be attributed to the action of this stimulator compound. Plants sprayed with fulvic acid resulted in a considerable increase in the maximum output of essential oil per square meter. The first harvest resulted in 1.87 ml, whereas the second produced 2.11 ml.

Regarding the impact of sandponic growth medium, the findings revealed that planting under silica caused the highest significant values of essential oil percentage. These determinations were 1.20% and 1.38% for the first and second harvests, respectively. The highest concentrations of essential oil per square meter were found in silica and the mixture of sand and silica, with no statistically significant differences between them. These values were 1.77 ml and 2.10 ml for silica in the first and second cuts, respectively, and 1.81 ml and 1.91 ml in the first and second cuts for the mixture between sand and silica.

The responses of essential oil yield to factor interactions were as follows: The significant top-volatile oil yield in both cuts came from plants grown in silica and sand with silica and sprayed with fulvic, with no statistically significant differences. These values were 2.10 ml and 2.24 ml for silica and fulvic acid in the first and second cuts and 2.09 ml and 2.60 ml for the sand-silica mixture using the fulvic spray in the first and second cuts in that order.

The chemical composition of creeping thyme essential oil revealed that it was composed of thymol (41.80-53.45%), o-cymene (21.58-32.29%), and γ-terpinene (7.97-14.14%), and its components were affected by various treatments. Thymol, an important compound responsible for the spicy flavor of creeping thyme, was found in the highest concentrations in silica (53.45%) and in plants grown in silica plus spraying with fulvic acid (49.56%). The existing influence of agronomic treatments on secondary metabolites of medicinal and aromatic plants was in the same line with El-Mahrouk *et al*. (2018) [12], Hamed E.S (2018) [13], Abd El-Aleem *et al*. (2021) [14], Abd El-Wahab *et al*. (2022) [15] and Hanafy *et al*. (2022) [16].

Table 4: Effect of fulvic acid, growth media, and their interaction on essential oil percentage (mean values of the two successive seasons).

Treatments	**1st cut**				**2nd cut**			
Fulvic acid	**Growth media**				**Growth media**			
	Sand	**Silica**	**Sand + silica**	**Mean**	**Sand**	**Silica**	**Sand + silica**	**Mean**
Without	0.97	1.15	1.10	1.07	1.00	1.45	1.21	1.22
With	1.03	1.25	1.10	1.13	0.94	1.31	1.36	1.20
Mean	1.00	1.20	1.10		0.97	1.38	1.29	
LSD 5%								
Fulvic acid	0.02				0.01			
Growth media	0.03				0.01			
Interaction	0.04				0.01			

Table 5: Effect of fulvic acid, growth media, and their interaction on essential oil yield (ml/m^2) (mean values of the two successive seasons).

Treatments	**1st cut**				**2nd cut**			
Fulvic acid	**Growth media**				**Growth media**			
	Sand	**Silica**	**Sand + silica**	**Mean**	**Sand**	**Silica**	**Sand + silica**	**Mean**
Without	1.38	1.44	1.53	1.45	1.47	1.96	1.21	1.55
With	1.42	2.10	2.09	1.87	1.50	2.24	2.60	2.11
Mean	1.40	1.77	1.81		1.49	2.10	1.91	
LSD 5%								
Fulvic acid	0.23				0.24			
Growth media	0.27				0.29			
Interaction	0.38				0.41			

Table 6: Effect of interaction between growth media and fulvic acid on essential oil composition.

R.T.	**Compound**	**Treatments**					
		Sand	**Sand + fulvic acid**	**Silica**	**Silica + fulvic acid**	**Sand + silica**	**Sand + silica + fulvic acid**
3.83	2-Thujene	2.80	1.98	1.70	1.50	1.81	2.89
3.98	α-pinene	2.38	2.18	2.20	1.88	2.47	2.75
4.30	Camphene	0.12	0.20	0.26	0.13	0.26	0.29
4.69	p-Mentha-1(7),3-diene	0.11	-	-	1.22	-	0.12
5.09	1-Octen-3-ol	2.22	1.84	1.50	1.67	2.81	1.91
5.54	α-Terpinene	2.51	1.26	1.39	-	1.79	2.20
5.79	o-Cymene	21.84	32.29	21.58	26.31	29.63	29.63
6.39	γ-Terpinene	14.14	7.97	10.46	9.17	11.59	11.01
6.87	Terpineol	0.84	0.51	0.56	0.72	0.70	1.10
7.46	α-Linalool	1.06	3.89	1.16	1.51	1.29	1.19
8.07	1-Methyl-4-(methylethyl)-(E)-2-cyclohexenol	0.05	-	-	-	0.05	0.05
8.51	Alcanfor	-	0.24	0.12	0.07	0.13	0.12
9.10	Undecane	0.05	-	-	-	-	-
9.23	Borneol	-	1.03	1.33	0.95	1.34	1.50
9.27	Terpinen-4-ol	1.14	-	-	-		
10.14	Anisole, 2-isopropyl-5-methyl	1.02	1.50	1.80	2.76	1.92	0.88
10.34	2-Isopropyl-1-methoxy-4-methylbenzene	0.61	-	-	-	-	-
11.19	Bornyl acetate	-	0.09	0.08	0.09	0.07	0.07
11.20	Geranyl isovalerate	0.06	-	-		-	-
12.04	Thymol	46.81	43.90	53.45	49.56	42.29	41.80
12.46	Carvacrol	1.61	0.26	1.66	1.76	1.20	1.92
13.09	Nerol acetate	-	0.16	-	-	0.06	-
13.92	Caryophyllene	0.28	0.25	0.32	0.24	0.42	0.29
15.83	Cadina-1(10),4-diene	0.05	0.08	0.07	0.09	0.15	0.14

17.23	Caryophyllene oxide	0.05	-	0.06	0.07	0.02	0.04
18.43	.tau.-Cadinol	0.05	0.08	-	0.09	-	0.10
Total identified components		99.80	99.71	99.70	99.79	100.00	100.00
Total hydrocarbon compounds		44.28	46.21	37.98	40.54	48.12	49.32
Total oxygenated compounds		55.52	53.5	61.72	59.25	51.88	50.68

R.T. = Retention time

The following reasons may be responsible for spraying fulvic acid's effect on increasing plant growth, yield, and active constituents: it positively impacts plant DNA and RNA, increases enzyme activity, acts as a catalyst in plant respiration, enhances cell division and elongation, aids chlorophyll synthesis, and is a rich source of amino acids. All of these benefits come from the fact that it stimulates the metabolism of plants. In addition, it improves the permeability of the cell members, restores electrochemical balance, raises the plant's capacity to take in oxygen, boosts the plant's resistance to drought, stops it from wilting, and protects against biotic stress. These findings are in line with those obtained by Aslantürk (2019) [17] in the study of onions, Justi *et al*. (2019) [18] in the study of coffee seedlings, Samavatipour *et al*. (2019) [19] on dill, Fang *et al*. (2020) [20] on Osti's tree peony, Hemmati *et al*. (2021) [21] on aloe, and Ali *et al*., (2022) [5] on the Damask rose.

Thyme needs low water requirements. So, the increase in fresh weight production that was reported to be associated with the media of sand with silica has been brought about by several different factors. These media can improve the circumstances for rooting, provide anchorage for the root system, provide plants with water and nutrients, and provide an appropriate environment for supplying aeration to the roots. They have a physical structure capable of maintaining a favorable balance between air and water storage during irrigation episodes and between them, which helps avoid root hypoxia and drought stress. These are important for the plant. In accordance with Baudoin *et al*. (2013) [22], Xego *et al*. (2017) [23], Patil *et al*. 2020 [24], and Abd El-Wahab *et al*. (2022) [15], the authors found variations in growth and yield that were caused by differences in the culture media used in hydroponics.

Conclusion

According to the results of the current hydroponic experiment on creeping thyme, for producing the most profitable fresh weight and other suitable characteristics such as dry mass and essential oil yield, it is recommended that the sandponic be filled with an equal amount of silica and sand. In addition, the plants should be sprayed with fulvic acid one month after transplanting and again after each cut.

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