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IMPACT OF BIO-FERTILIZER AND HUMIC ACID APPLICATIONS ON PRODUCTIVITY AND SOME BIOCHEMICAL CHARACTERISTICS OF THYMUS VULGAIS GROW IN SANDY SOIL.

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ABSTRACT

This research was conducted at Agriculture Faculty Nursery at Mansoura University, during the two seasons of 2022/2023. In order to study the effect of humic acid (HA) and arbuscular mycorrhizal fungi (AMF) and their interactions on vegetative growth, essential oil and chemical composition of *Thymus vulgais*. Plants were irrigated with (HA) as bio-stimulant applied monthly at the concentrations of (0, 0.5, 1 and 2%) grown in pots containing both sandy soil with or without (AMF). The results showed that added (AMF) to the soil led to significantly increase in the studied traits such as, growth parameter, yield, chlorophyll content, total carbohydrates and essential oil percentage. Specifically, (AMF) combined with (HA) at 1% resulted in a significant increase in fresh weight (16-46%), chlorophyll a (84%), carbohydrate (4-32%), and oil yield (75-80%) as compered with (AMF) without humic acid. Furthermore, the application of (HA) at 1% also influenced the composition of thymus oil. The results cited above concluded that (AMF) and humic acid increase both growth and essential oil production in plants which enhanced its commercial value.

Keywords: *Thymus vulgaris*, bio-fertilizer, Arbuscular Mycorhizal fungi, Humic acid.

INTRODUCTION

Thyme (*Thymus vulgaris* L.), an aromatic perennial subshrub in the Lamiaceae (labiatae) family, has long been recognized for its therapeutic properties **(Kucukaydin *et al*., 2020)**. Thyme is used as a traditional and folkloric medicine for cough, diabetes, and chest infections (bronchitis, pharyngitis, whooping cough) according to **(Askary *et al*., 2018)** Furthermore, the volatile essential oil (EO) extracted from thyme aerial parts exhibits expectorant analgesic, antibacterial, antispasmodic, antiseptic, and antifungal properties. **(Amani Machiani *et al*., 2021 and Pavela *et al*., 2018)**.

Humic acid (HA) is a natural organic polymer compound formed by the decay of organic matter in soil that can enhance plant productivity and quality **(Eshwar *et al*., 2017)**. One of the most notable benefits of humic acid is being rich in different nutrients, including calcium, zinc, sodium, potassium, magnesium, iron, copper, and other elements **(Ihsanullah and Bakhashwain, 2013)**. Potassium humate is a potentially enhancing growth, yield and nutritional status by improving the physical and chemical characteristics of the soil and dynamics of nutrients **(Kumar *et al*., 2013)**. Additionally, HA compounds enhanced the length of the roots and shoots, water content in the leaves, photosynthesis and enzymatic activity under various conditions of stress **(Kaya *et al*., 2018; Sheteiwy *et al*., 2017)**.

Arbuscular mycorrhizal fungi (AMF) are important ecological consituents of soils, making symbiotic relationships with about 80% of vascular plants in terrestrial ecosystems **(Smith and Read, 2008; Gao *et al*., 2019)**. AMF are recognized as an important component of the rhizosphere of many species of medicinal plants in their natural environments **(Assis *et al*., 2020)**. AMF are one of the most important types of bio-fertilizers, through improving the ecological balance of the soil and increase yield **(Weisany *et al*., 2016)**. Moreover, plant nutrient mineralization, nutrient cycling, and degradation of soil organic matter are all significantly affected by AMF symbiotic relationships **(Jansa *et al*., 2019)**. AMF can improve nutrition of plants and the quality of essential oils **(Nell *et al*., 2009).**

The aim of this study was to investigate the effect of AMF and humic acid on improving plant growth, oil yield and its main components thus, providing growers with useful information about the response of thyme plants to mycorrhiza and humic acid.

2. MATERIALS AND METHODS

2.1. Plant material and growth conditions

A pot experiment was performed at Agriculture Faculty Nursery at Mansoura University, during the two seasons of 2021/2022 and 2022/2023. The experimental soil comprised sand (76%), silt (13%), clay (11%), organic matter (1.68%), nitrogen (4.5ppm) and phosphorus (9.45ppm). The sandy soil was distributed among pots (8 kg pot^{-1}), which were 250 mm in diameter and 350 mm in height. The treatments were arranged in complete randomized block design with three replicates (six pots/replicate). Soils were inoculated with AMF (+AMF) or uninoculated (-

AMF). The AMF used in this study was originally provided by Prof. Dr. Gamal Abdel Fattah Ouf, Faculty of Science, Mansoura University, Egypt. It was originally isolated from rhizosphere soil of onion (*Allium cepa)* using the wet sieving and decanting technique **(Gerdemann and Nicolson, 1963)**, and then identified by the author. Thyme seedlings (13 - 15 cm height) were acquired from Medicinal and Aromatic Plants Research Farm, El-Kanater el-khairia, El-Kalubia Governorate, ARC, Giza, Egypt. The seedlings were transplanted into the pots on the 21 th and 25 th March during 2022 and 2023 seasons, respectively. Then one-two seedling was placed into the hole. Then, 5g of AMF inoculum (containing of soil containing 50 spores gram $^{-1}$) were added. The inoculum was buried under the soil suface (30-50 mm). Humic acid (HA) treatment as potassium humate (85% humate and 15% potassium) was used as a source of HA, which dissolved in tap water to prepare the levels of (0, 0.5, 1.5 and 2%). The treatments were irrigated with HA during the vegetative and flowering. The first application of humic acid was carried out 30-days after planting and repeated at 30-days interval during the growth period. Control plants were treated with tap water. Plants were irrigated with tap water as needed.

The experiment included 10 treatments as follow:

1. Control
2. 0.5% Humic acid
3. 1% Humic acid
4. 1.5% Humic acid
5. 2% Humic acid
6. Mycorrhiza
7. 0.5% Humic acid + Mycorrhiza
8. 1% Humic acid + Mycorrhiza
9. 1.5% Humic acid + Mycorrhiza
10. 2% Humic acid + Mycorrhiza.

Before transplanting, the chemical and physical properties of used soil in this study were determined by Soil Fertility Laboratory in Mansoura in Agricultural Research Center as shown in **Table (1)** .

Table 1: The physical and chemical characteristics of the soil sample.

Physical characteristics								
Soil texture			**Sandy soil**	**pH**	**EC mmhos/cm**	**Organic matter %**	**Organic carbon %**	
Clay%	**Silt%**	**Sand%**						
11	**13**	**76**		**7.87**	**0.42**	**1.68**	**0.75**	
Chemical characteristics								
Soluble Cations (meq/100gm soil)				Soluble Anions(meq/100gm soil)				
Ca^{++}	Mg^{++}	K^{+}	Na^{+}	Cl^{-}	SO_4^{--}	HCO_3	Available N (ppm)	Available P (ppm)
0.22	0.26	0.05	1.63	0.33	1.34	0.50	4.5	9.45

2.2. Growth measurements:

The plants were harvested twice (1st and 2nd cuts) through cutting the aerial parts of each plant at 8-10 cm above the soil surface. The two cuts were taken on 28th May and 7 August in first and second seasons, respectively the data of vegetative growth parameters are as follows: plant height (cm), fresh and dry weight of herb/plant (g), as well as pigment content, carbohydrates content, oil% and essential oil % and its comosation as chemical analysis.

2.3. Mycorrhizal colonization estimation

Fresh roots from mycorrhiza treatments were harvested, rinsed in tap water to remove any remaining soil particles, and then immersed in 10% KOH. The roots were then stained with 0.05% trypan blue (Sigma) in lactophenol for 15 minutes at 90°C. In order to measure mycorrhizal colonization, thirty stained root segments were arranged on slides for each treatment, and viewed under a 40x magnification microscope **(Phillips and Hayman, 1970)**. Based on the presence of AMF structures in the root segments, Frequency of root colonization (F), intensity of mycorrhizal colonization (M%) and arbuscular development(A%) were measured.

2.4. Chemicals analysis:

2.4.1. Photosynthetic pigments content

Chemical analysis was carried out in the laboratory of Botany Dept. Fac. Sci., Mansoura Univ. The plant photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) were determined spectrophotometrically as suggested by **Arnon (1949)** and modified by **Vimala and Poonghuzhali (2014)**. The extract was compared to a blank of pure 80% aqueous acetone at three wave lengths of 480, 644, and 663 nm. To estimate the carotenoid content, the same acetone extract was measured at 480 nm according to **Kirk and Allen (1965).**

2.4.2. Total carbohydrate content

Total carbohydrate content in dried herb sample was determined according to **Hedge and Hofreiter's (1962)**.

2.4.3. Essential oil percentage

Essential oil % in thyme dry herb was determined according to **British Pharmacopoeia (2002).**

2.4.4. Essential oil composition:

Essential oil composition: Gas liquid chromatography (GLC) analysis was used to analyze the essential oil samples and carried out in Laboratory of, Horticulture Research Institute. The obtained chromatogram and report at GC analysis for each sample were analyzed to calculate the percentage of main components of the volatile oil according to **Guenther and Joseph (1978)**.

2.5. Statistical analysis

Data were presented as the mean (± SE) of three replicates. Analyses were done using ANOVA procedure, and least significant difference (LSD) among means were calculated at $P \leq 0.05$ **Steel and Torrie (1980).**

3. RESULTS AND DISCUSSIONS

3.1. Effect on Vegetative Growth

Results illustrated in **Tables (2)** and **Figure (1 and 2)** showed that the effect of humic acid and AMF on the growth and yield characters demonstrated that plant height, root length and fresh and dry weights were significantly greater in inoculated mycorrhizal thyme plants as compared to control treatment. Regarding combination treatments, M. +1% HA and M. +1.5% HA recorded the highest values of previously mentioned parameters as compared with other treatments at two cuts of two seasons

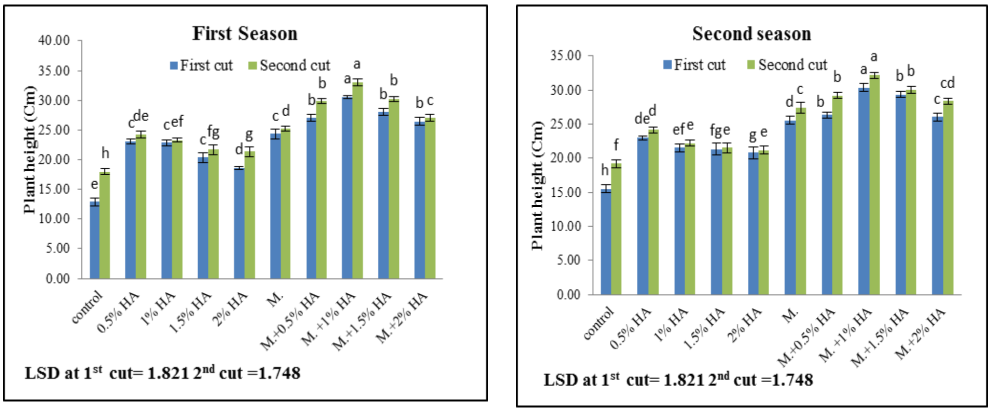

Figure 1: Impact of HA and AMF treatments on plant height of thyme (Thymus vulgaris L.) plant during two cuts in the two seasons.Data are expressed as means±SE(n=3)

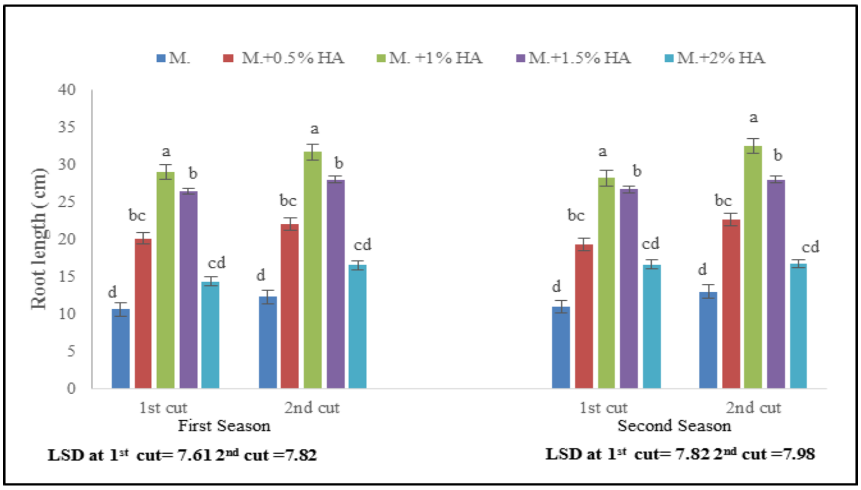

Figure 2: Effect of HA and AMF treatments on root length of thyme (*Thymus vulgaris* L.) plant during two cuts in the two seasons. Data are expressed as means± SE(n=3).

Table 2: Effect of HA and AMF treatments on fresh and dry weights / plant (g) of thyme (*Thymus vulgaris* L.) plant during two cuts in the two seasons.

Treatment	First season		Second season	
	1st cut	**2nd cut**	**1st cut**	**2nd cut**
	Fresh weight of herb g/plant			
Control	13.56±0.29^{g}	19.30±0.37^{g}	13.10±0.36^{g}	21.23±0.64^{h}
0.5% HA	20.33±0.49^{de}	32.96±0.33^{d}	19.87±0.59^{d}	34.70±0.73^{f}
1% HA	19.93±0.52^{de}	33.43±0.44^{d}	19.53±0.14^{d}	33.03±0.49^{f}
1.5% HA	19.33±0.69^{e}	30.03±0.91^{e}	16.30±0.20^{e}	25.26±0.71^{g}
2% HA	15.07±0.60^{f}	28.13±0.69^{f}	16.10±0.40^{f}	23.73±0.55^{g}
M.	21.17±0.52^{cd}	34.63±0.72^{d}	20.00±0.57^{d}	38.80±0.83^{e}
0.5% HA.+ M.	22.53±0.29^{b}	39.93±0.52^{b}	21.40±0.51^{b}	44.63±0.62^{c}
1% HA + M.	24.53±0.29^{a}	44.50±0.55^{a}	27.97±0.12^{a}	56.73±0.80^{a}
1.5% HA + M.	23.20±0.23^{b}	41.60±0.81^{b}	23.80±0.1^{b}	49.25±0.85^{b}
2% HA + M.	22.30±0.11^{bc}	37.43±0.65^{c}	20.16±0.29^{c}	43.63±0.73^{d}
LSD 0.05	1.305	1.859	1.859	2.090
	dry weight of herb g/plant			
Control	6.90±0.05^{e}	8.93±0.86^{e}	6.20±0.83^{f}	8.73±0.17^{h}
0.5% HA	7.86±0.57^{d}	10.40±0.11^{bcd}	8.96±0.18^{bc}	12.66±0.48^{e}
1% HA	7.66±0.08^{de}	10.10±0.20^{cde}	8.03±0.14^{cd}	11.63±0.31^{ef}
1.5% HA	7.57±0.21^{de}	10.03±0.14^{cde}	7.20±0.20^{de}	10.43±0.231^{fg}
2% HA	7.00±0.11^{e}	9.30±0.30^{de}	6.83±0.08^{ef}	9.80±0.28^{gh}
M.	8.00±0.30^{d}	11.06±0.43^{bc}	9.33±0.17^{b}	15.47±0.48^{d}
0.5% HA + M.	9.83±0.29^{bc}	13.70±0.11^{a}	10.50±0.11^{a}	17.50±0.28^{bc}
1% HA + M.	12.53±0.52^{a}	14.26±0.69^{a}	11.30±0.15^{a}	19.10±0.60^{a}
1.5% HA + M.	10.13±0.29^{b}	13.80±0.17^{a}	11.00±0.35^{a}	18.90±0.76^{ab}
2% HA + M.	9.10±0.20^{c}	11.53±0.51^{b}	9.73±0.17^{b}	16.73±0.42^{cd}
LSD 0.05	0.773	1.286	0.934	1.306

*HA: Humic Acid, ** M.: Mycorrhizal fungi; Data are means ± SE; different letters within the same column indicate significant differences among means according to LSD at $p\leq0.05$.

As the role of humic acid and mycorrhizal fungi on thyme plants, potassium humate contains many elements necessary to the plant life development **(El-Sharkawy and Abdel-Razzak, 2010).** Humic acid is a natural polymer organic compound and is one of the major components of humus and containing most of known trace minerals necessary to plant growth **(El Ziat *et al*., 2018**). Humic acid application improved soil physical and chemical characteristics which increased the nutrient mineral adsorption **(Bakry *et al*., 2015)**.In addition, **Verlinden *et al*. (2010)** reported that the application of humic acid led to increase the plant's production.

As well as mycorrhiza play an important role in vegetation restoration because of symbiosis with plant root; they can facilitate mineral absorption by host plant, stability and improve soil structure, affect the population structure and preserve species diversity **(Bothe *et al*., 2010).** AMF effectively increased plant growth and dry weight, presumably because AMF quickly spread to plant roots, forming high root colonization **(Ingleby *et al*., 2007)**. The increased root length of the mycorrhizal inoculated plants which is confirmed by other studies **(Prasad *et al*., 2017)**, might have been due to the higher uptake of elements .The increased root length by AMF inoculation was attributed to the enhanced element uptake **(Sohn *et al*., 2003 and Sheikh-Assadi *et al*., 2023).** The effectiveness of AMF in increasing plant growth is that AMF can supply P and N nutrients by forming external hyphae so that they can be available to plants **(Muleta, 2017)**. Moreover, these results are in accordance with **Prameswari *et al*. (2021) and Sheikh-Assadi *et al*. (2023).** The treatments of mycorrhizal inoculation and humic acid significantly increased plant growth parameters including plant height and fresh herb and dry weights. The positive effects of mycorrhizal inoculation and humic acids on plant growth were also confirmed by prior studies (**Nikbakht *et al*., 2008; El-khateeb *et al*.; 2011, Habib, 2021).**

3.2. Colonization of thyme roots by arbuscular mycorhizal fungi

The root colonization by arbuscular fungus was monitored using frequency (F %), intensity (M %) of mycorrhizal colonization and arbuscular development (A %). These indices of AMF colonization in thyme roots were significantly increased by humic acid concentration **(Table 3).** Treatment with 1% humic acid increased mycorrhizal colonization however, these measure gradually declined with increasing humic acid concentration.

3.3. Photosynthetic pigments content:

Data presented in **Table 4** showed that the highest content of all determined photosynthetic pigments; chlorophyll a, chlorophyll b, and carotenoids were significantly increased in M.+1% HA and M.+1.5%HA herb treatments as compared with other treatments or control at the two cuts of the two seasons. On the other hand 0.5% HA treatment showed the highest value as compared to the humic acid treatments without mycorhizal inoculation

Table 3: Mycorrhizal colonization levels (%) of mycorrhizal (+AMF) and non mycorrhizal (-AMF) thyme plants under diffenrent concentration of humic acid during two cuts in the two seasons (2022-2023).

Treatment	Mycorrhizal colonization levels (%)					
	F	M	A	F	M	A
	First season					
	1^{st} cut			2^{nd} cut		
Control	--	--	--	--	--	--
0.5% HA	--	--	--	--	--	--
1% HA	--	--	--	--	--	--
1.5% HA	--	--	--	--	--	--
2% HA	--	--	--	--	--	--
M.	84.11±0.48^{a}	47.4±0.43^{b}	16.40±0.83^{a}	100.00±0.00^{a}	52.23±1.46^{b}	11.76±0.72^{ab}
0.5% HA+M.	70.56±2.42^{b}	45.44±1.09^{b}	16.33±1.45^{a}	84.28±0.49^{b}	54.00±2.01^{b}	11.83±1.09^{ab}
1% HA+M.	83.94±0.53^{a}	52.33±1.39^{a}	12.07±1.0^{b}	100.00±0.00^{a}	65.33±0.71^{a}	12.50±0.86^{ab}
1.5% HA+M.	69.65±1.63^{b}	38.23±0.95^{c}	15.50±1.32^{ab}	100.00±0.00^{a}	51.96±1.01^{b}	10.7±0.21^{b}
2% HA +M.	52.33±1.45^{c}	38.06±1.79^{c}	16.06±1.09^{a}	83.27±0.43^{c}	45.53±1.29^{c}	13.73±0.72^{a}
LSD 0.05	4.70	3.84	3.67	0.92	4.31	2.45
	Second season					
	1^{st} cut			**2^{nd} cut**		
Control	--	--	--	--	--	--
0.5% HA	--	--	--	--	--	--
1% HA	--	--	--	--	--	--
1.5% HA	--	--	--	--	--	--
2% HA	--	--	--	--	--	--
M.	83.11±0.58^{a}	46.33±0.88^{c}	13.43±0.35^{b}	100.00±0.00^{a}	89.9±1.06^{a}	10.76±0.14^{b}
0.5% HA +M.	70.39±2.26^{b}	56.53±0.091^{b}	18.50±0.36^{a}	100.00±0.00^{a}	91.50.81^{a}	15.23±0.43^{a}
1% HA +M.	83.61±0.20^{a}	56.83±0.97^{b}	11.27±0.37^{c}	100.00±0.00^{a}	76.56±0.88^{b}	16.53±0.61^{a}
1.5% HA +M.	66.39±0.45^{b}	65.40±0.7^{a}	11.03±0.15^{c}	100.00±0.00^{a}	76.17±1.24^{b}	16.06±1.21^{a}
2% HA +M.	44.33±2.33^{c}	36.66±1.76^{d}	10.9±0.17^{c}	84.27±0.64^{b}	45.53±0.74^{c}	10.6±0.45^{b}
LSD 0.05	4.71	3.49	0.93	0.90	3.05	2.12

*HA: Humic Acid, ** M.: Mycorrhizal fungi *** F: frequency ****M: intensity ***** A: development of arbuscular mycorhizal fungi; Data are means ± SE; different letters within the same column indicate significant differences between means according to LSD at $p \leq 0.05$.

Table 4: Effect of humic acid and mycorrhizal fungi on pigments content (mg g^{-1} d wt) of thyme (*Thymus vulgaris* L.) plant during two cuts in the two seasons (2022-2023).

Treatment	First season					
	1st cut			2nd cut		
	Pigment content (mg g-1 d wt)					
	Chla	**Chl b**	**Carotenoids**	**Chla**	**Chl b**	**Carotenoids**
Control	0.888±0.019^{d}	0.556±0.009^{f}	0.283±0.010^{de}	0.919±0.072^{e}	0.602±0.080^{f}	0.424±0.011^{e}
0.5% HA	0.940±0.013^{cd}	0.686±0.050^{de}	0.353±0.008^{cd}	1.158±0.008^{c}	0.754±0.015^{cd}	0.481±0.017^{cde}
1% HA	0.903±0.014^{d}	0.601±0.077^{ef}	0.320±0.003^{cde}	1.120±0.007^{cd}	0.75±0.015^{de}	0.462±0.00^{cde}
1.5% HA	0.898±0.013^{d}	0.594±0.042^{ef}	0.218±0.094^{cde}	1.021±0.082^{de}	0.631±0.019^{ef}	0.440±0.006^{de}
2% HA	0.870±0.012^{d}	0.547±0.042^{f}	0.218±0.094^{e}	1.001±0.056^{de}	0.623±0.035^{ef}	0.418±0.043^{e}
M.	1.011±0.018^{c}	0.780±0.012^{cd}	0.399±0.005^{bc}	1.166±0.020^{c}	0.828±0.036^{bc}	0.512±0.067^{bcd}
0.5% HA+M.	1.209±0.020^{b}	0.865±0.022^{abc}	0.511±0.006^{a}	1.222±0.039^{bc}	0.921±0.004^{ab}	0.545±0.018^{abc}
1% HA+M.	1.379±0.052^{a}	0.934±0.027^{a}	0.567±0.045^{a}	1.441±0.039^{a}	0.961±0.021^{a}	0.611±0.004^{a}
1.5% HA+M.	1.303±0.038^{a}	0.903±0.049^{ab}	0.535±0.002^{a}	1.321±0.049^{ab}	0.939±0.016^{a}	0.579±0.003^{ab}
2% HA +M.	1.150±0.030^{b}	0.795±0.007^{bcd}	0.473±0.044^{e}	1.207±0.009^{bc}	0.872±0.044^{ab}	0.528±0.031^{abc}
LSD	0.078	0.119	0.107	0.136	0.104	0.085
	Second season					
Control	0.915±0.006^{d}	0.540±0.022^{f}	0.282±0.024^{f}	0.892±0.006^{f}	0.599±0.025^{e}	0.302±0.007^{e}
0.5% HA	0.996±0.006^{cd}	0.692±0.019^{de}	0.359±0.010^{d}	1.122±0.020^{e}	0.750±0.023^{bcd}	0.363±0.009^{cd}
1% HA	0.947±0.015^{cd}	0.663±0.028^{de}	0.331±0.006^{de}	1.044±0.011^{e}	0.698±0.013^{cde}	0.338±0.006^{de}
1.5% HA	0.930±0.011^{cd}	0.604±0.054^{ef}	0.319±0.003^{def}	0.947±0.013^{f}	0.736±0.057^{bcde}	0.329±0.003^{de}
2% HA	0.928±0.004^{cd}	0.767±0.038^{ef}	0.302±0.003^{ef}	0.885±0.008^{f}	0.630±0.077^{de}	0.309±0.002^{e}
M.	1.030±0.085^{c}	0.544±0.234^{cd}	0.353±0.021^{d}	1.218±0.045^{cd}	0.787±0.047^{bc}	0.398±0.013^{c}
0.5% HA+M.	1.263±0.017^{b}	0.818±0.008^{bc}	0.481±0.003^{b}	1.270±0.008^{c}	0.804±0.024^{abc}	0.491±0.002^{b}
1% HA+M.	1.519±0.012^{a}	0.945±0.035^{a}	0.580±0.007^{a}	1.556±0.031^{a}	0.941±0.008^{a}	0.587±0.002^{a}
1.5% HA+M.	1.418±0.082^{a}	0.892±0.051^{ab}	0.569±0.021^{a}	1.384±0.049^{b}	0.857±0.032^{ab}	0.551±0.027^{a}
2% HA +M.	1.239±0.008^{b}	0.761±0.039^{cd}	0.419±0.011^{c}	1.128±0.057^{de}	0.796±0.093^{bc}	0.476±0.017^{b}
LSD	0.114	0.107	0.040	0.091	0.148	0.036

*HA: Humic Acid, ** M.: Mycorrhizal fungi; Data are means ± SE; different letters within the same column indicate significant differences between means according to LSD at $p \leq 0.05$.

Humic acid can improve plant growth directly by accelerating the proteosynthesis, increasing water and nutrient uptake and yields of plants **(Panda, 2006)**. Also, it increases the chlorophyll content of green plants, and hence improve photosynthesis **(Nardi *et al*., 2002; Bettoni *et al*., 2014)**. Additionally, humic acid may have contributed to the improvement of photosynthesis activity and increase in total chlorophyll content by promoting amino acid synthesis, accelerating nitrogen and nitrate absorption, increasing nitrogen metabolism, and promoting protein production **(Alfatlawi and Alrubaiee, 2020)**.

From our results, it can be stated that the mycorrhizal inoculation treatment was the most effective one for promoting the synthesis and accumulation of three photosynthetic pigments. These results are in accordance with **Oyun *et al*. (2010) and El-khateeb *et al*. (2011).** Plants that inoculated with Mycorrhizal usually have greater photosynthetic pigments content and chlorophyll fluorescence efficiency, and so attain more tolerance to environmental stresses **(Battah *et al*., 2021).** It was seen that AMF colonization enhanced chlorophyll content of dill **(Weisany *et al*., 2015)** and thereby increased photosynthesis in plants inoculated with mycorrhiza, which improves the export of triose phosphate to the cytoplasm. Thus, the Calvin cycle activity will rise and intensify the production of primary metabolites as well as precursors of secondary metabolites **(Kaschuk *et al*., 2009).**

3.4. Carbohydrates content

The obtained data in **Table 5** indicated that total carbohydrates content increased in case of HA 1% + M. and HA 1.5% + M. in two cuts of both seasons as compared to control, whereas, in uninoculated and sprayed with 0.5% HA gave the highest total carbohydrates content as compared to each individual humic acid treatments.

Table 5: Effect of humic acid and mycorrhizal fungi treatments on total carbohydrates content of thyme (*Thymus Avulgaris* L.) plant during two cuts in the two seasons.

Treatment	First season		Second season	
	1st cut	2nd cut	1st cut	2nd cut
	Total carbohydrates (mg/g dry wt)			
Control	83.54±1.80g	85.22±2.38g	82.30±0.59g	83.07±0.47h
0.5% HA	92.65±0.59cd	110.89±1.25e	91.75±1.16de	94.45±0.78e
1% HA	90.01±0.56de	109.09±0.81e	90.85±2.21def	92.20±0.78ef
1.5% HA	87.33±0.88ef	95.58±0.81f	87.69±0.38ef	89.50±1.19fg
2% HA	85.67±0.59fg	88.14±0.59g	86.79±0.59f	87.25±1.47g
M.	94.45±1.02c	116.75±1.78d	92.88±0.97d	107.07±1.19d
0.5% HA + M.	109.54±2.65b	120.35±0.98bc	112.02±1.16b	117.65±1.57c
1% HA + M.	121.71±0.80a	126.66±0.98a	121.02±1.25a	124.70±0.67a
1.5% HA + M.	120.58±0.59a	122.60±0.81b	118.78±1.03a	121.03±1.62b
2% HA + M.	95.13±0.39c	117.65±0.59cd	100.53±3.04c	109.77±0.81d
LSD 0.05	3.41	3.30	4.31	3.29

*HA: Humic Acid, ** M.: Mycorrhizal fungi; Data are means ± SE; different letters within the same column indicate significant differences between means according to LSD at p≤0.05.

The combined treatment of mycorrhizal fungi and humic acid increased the total carbohydrates content as compared to control. **Bakry *et al*. (2014)** reported that HA enhances the absorption of Fe and P and other nutritional elements, and then improves nutritional status of plant. Thereby, increasing the photosynthetic pigments and the photosynthetic machinery and thereby increasing the carbohydrate. The positive increments in the total carbohydrates content due to mycorrhizal treatment is in harmony with **El-Khateeb *et al*. (2010a)**, on *chamaedorea elegans*, who found that total carbohydrates content increased significantly with the inoculation with mycorrhizal fungi. Moreover, **Pedone-Bonfim *et al*. (2018)** reported that *Mimosa tenuiflora* seedlings showed the greater photosynthetic performance and content of soluble carbohydrates and secondary metabolite as compared to non-mycorrhizal plants. Also, **El-Khateeb *et al*. (2010b)** on *Calia secundiflora*, found that total carbohydrates content increased with humic acid and mycorrhizal inoculation.

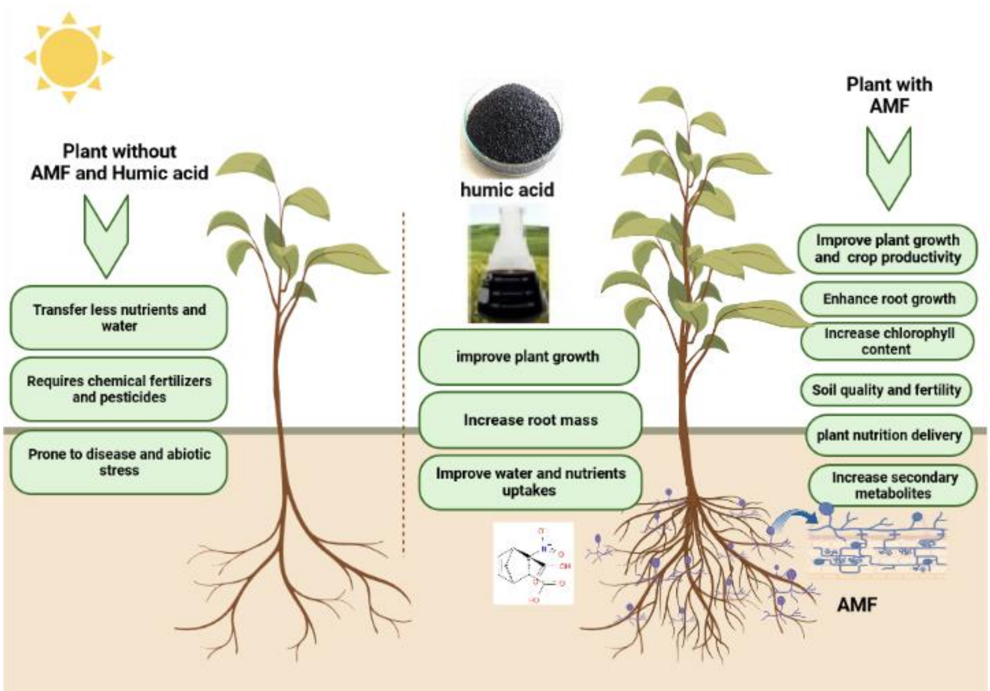

Figure 3: a schematic diagram illustrating the various ways in which plants react to humic acid and mycorrhizal fungi (AMF) in both presence and absence. Without AMF and HA, plants have undeveloped root systems that make hard for them to absorb water and nutrients from the soil. They also need chemical pesticides and fertilizers to grow. Plants that possess both AMF and HA have a symbiotic relationship with Arbuscular mycorrhizal fungi (AMF) in their roots, which enhances plant growth and productivity. Moreover, raise the concentration of secondary metabolites and chlorophyll.

3.5. Essential oils percentage

The data for essential oil percentage per plant are presented in **Table (6).** It is clear that, thyme plants inoculated with mycorrhizal significantly improved the essential oil percentage per plant as compared to uninoculated plants in the two cuts of both seasons. The combination effect between mycorrhizal and humic acid indicated that mycorrhizal fungi and 1% or 1.5% humic acid, gave the highest valus of essential oils perecentage as compared to other treatments or control these results were in the same line in the two cuts of the two seasons.

Table 6: Effect of humic acid and mycorrhizal fungi treatments on percentage of essential oil concentrations of thyme (*Thymus vulgaris* L.) plant during two cuts in the two seasons.

Treatment	First season		Second season	
	1st cut	2nd cut	1st cut	2nd cut
Control	0.21±0.008[g]	0.24±0.011[g]	0.22±0.012[f]	0.24±0.003[h]
0.5% HA	0.30±0.011[de]	0.32±0.014[de]	0.34±0.012[d]	0.36±0.005[e]
1% HA	0.27±0.011[ef]	0.29±0.012[ef]	0.28±0.011[e]	0.30±0.011[f]
1.5% HA	0.26±0.011[f]	0.28±0.014[f]	0.27±0.011[e]	0.29±0.012[fg]
2% HA	0.25±0.012[fg]	0.26±0.005[fg]	0.25±0.011[ef]	0.26±0.008[gh]
M.	0.31±0.006[d]	0.36±0.012[d]	0.35±0.009[d]	0.40±0.006[d]
0.5% HA + M.	0.40±0.006[c]	0.44±0.012[c]	0.42±0.008[c]	0.49±0.006[c]
1% HA + M.	0.61±0.014[a]	0.65±0.014[a]	0.64±0.014[a]	0.70±0.009[a]
1.5% HA + M.	0.52±0.014[b]	0.009±0.56[b]	0.56±0.008[b]	0.58±0.008[b]
2% HA + M.	0.39±0.012[c]	0.42±0.14[c]	0.40±0.012[c]	0.47±0.011[c]
LSD	0.033	0.036	0.033	0.026

*HA: Humic Acid, ** M.: Mycorrhizal fungi; Data are means ± SE; different letters within the same column indicate significant differences between means according to LSD at p≤0.05.

Our findings are in agreement with those of other experiments. In a study on *Ocimum basilicum* by **Shafighi and Pazoki (2014)** who reported that an increase in the percentage of essential oils of medicinal plants by using mycorrhizal fertilizer. **Kapoor *et al*. (2002) and (2004)** proved that AM is the major role to improve the quantitative and qualitative yield of essential oils. **Noroozisharaf and Kaviani (2018)** examined the effects of various HA levels (control, 50, 75 and 100 g m^{-2}) on the chemical composition of *Thymus vulgaris* L. and pound that the essential oil concentration increased with the increase in HA levels as compared to the control. In addition, **Mohamed, *et al*. (2018)** reported that the growth and essential oil of Nepeta species time showed the highest essential oil concentration and yield in response to potassium humate.

3.6. Essential oil composition

The largest and most diverse group of secondary metabolites are terpenoids, which are primary constituents of essential oils **(Cox-Georgian *et al*. 2019)**. Essential oils are volatile lipophilic mixtures of secondary metabolites, consisting mostly of monoterpenes, sesquiterpenes, and phenylpropanoids, which often are used as flavours and fragrances, as well as antimicrobials, antioxidants and medicines **(Deans and Waterman 1993)**. It has been reported that the thyme essental oil is rich in oxygenated (60% mixture of thymol and carvacrol, linalool) and hydrocarbon monoterpenoids (p-cymene and γ-terpinene) **(Al-Asmari *et al*., 2017)**.

In this study, seventeen compounds, accounting for more than 99.16% of the total volatiles in most thyme samples in first cut of the second season were detected and identified **(Table 7)**. There were differences in oil composition as affected by humic acid and mycorrhizal fungi applications.

The predominant compounds present under all studied treatments were the *p*-cymene (69.00-91.35%), thymol (0.14-13.04 %), thymyl methyl ether (1.53-6.64 %), carvacrol (0.79-3.94 ،%) Carvacrol methyl ether (0.73-3.55 %), α- Pinene (0.19-2.06 %), α-Thujene (0.40-1.76 %) and 1, 8-Cineole (0.16-1.53 %) . Thyme plants inoculated with mycorrhiza and sprayed with 1% humic acid treatment gave an increase in the level of *p*-cymene (91.35%) and linalool (0.29%) relative to untreated control plants. The highest value in *p*-cymene was obtained from M. + 1% HA followed by individual 0.5% HA whereas the lowest value was recorded from individual mycorrhizal treatment.

These results are in line with those obtained by **Alireza and Maryamm (2018)** who reported that thirty-two volatile compounds were identified and these compounds were considerably affected by humic acid application. The highest percentage of thymol (74.15%), carvacrol (6.20%), p-cymene (4.24%), borneol (3.42%), trans-caryophyllene (1.70%) and cis- sabinene hydrate (1.35%) as major compounds were found in *Thymus vulgaris* by spring of 100 g m^{-2} humic acid. In addation, **Amani Machiani *et al*., (2021)** noted that the inoculation of thyme seedlings with *Funneliformis mosseae* improved quantity and quality through increasing the main constituents, such as thymol, p-cymene, and γ-terpinene, under drought stress conditions. Furthermore, **Bahadori *et al*. (2013)** reported that AMF inoculation treatments significantly increased the concentration of thymol in *T. daenensis* plants as compared to the control plants. AMF-inoculated plants could alter the number of peltate trichomes in *Ocimum basilicam* plants, and improve some essential oil components **(Copetta *et al*., 2006).**

Table 7: Essential oil components (%) of *Thymus vulgaris* plant as affected by humic acid and mycorrhizal fungi of the 2nd cut during the 2nd season.

Compound Name	Mol. Wt.	Class of compounds detected	% of compounds				
			Control	0.5% HA	M.	M. +1% HA	M. +1.5% HA
α-Thujene	136	Bicyclic monoterpenoids	1.03	1.76	2.81	0.55	0.40
α-Pinene	136	Bicyclic monoterpenoids	0.92	2.06	1.80	0.19	0.38
m-Cymene	134	Aromatic hydrocarbon	1.34	1.67	0.84	0.28	-
α-Methoxystyrene	134	Phenylpropenes	0.33	-	0.37	0.60	1.93
o-Cymene	134	Aromatic monoterpenoids	1.32	-	1.37	0.38	1.29
p-Cymene	134	Aromatic monoterpenoids	69.16	87.46	74.43	91.35	82.67
1,8-Cineole	154	Monoterpene cyclic ether	0.24	0.39	1.53	0.16	0.84
Linalool	154	Oxygenated Monoterpene	0.25	-	-	0.29	-
Camphor	152	Oxygenated Monoterpene	0.45	-	-	-	-
Endo-isocamphane	138	Bicyclic monoterpenoids	0.31	-	-	-	-
endoBorneol	154	Bicyclic monoterpenoids	0.55	0.45	1.51	-	-
Thymyl methyl ether	164	Aromatic monoterpenoids	6.64	1.79	1.53	1.69	6.04
Carvacrol methyl ether	164	Aromatic monoterpenoids	1.46	1.71	3.55	0.85	0.73
Thymol	150	Oxygenated Monoterpene	13.04	0.95	0.75	0.14	0.81
Carvacrol	150	Aromatic monoterpenoids	1.63	0.79	0.50	1.79	3.94
Isobornyl propionate	210	Bicyclic monoterpenoids	0.30	0.13	-	-	-
Caryophyllene	204	Hydrocarbon Sesquiterpene	0.16	-	0.10	0.14	-
99.13 99.16 91.09							

*HA: Humic Acid, ** M.: Mycorrhizal fungi *** R.T: Retenation time **** Mol. Wt.: Molecular weight.

4. CONCLUSIONS

The application of mycorrhizal inoculum in addition to HA spraying seems to be a valuable method for enhancing *Thymus vulgaris* growth, yield, and essential oil production under sandy soil conditions. The highest percentage of essential oil and herb yield were induced by the interaction of 1% HA with mycorrhizal inoclum.

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الملخص العربي

تأثير التسميد الحيوي وحامض الهيوميك على الإنتاجية وبعض الصفات الكيميائية لنبات الزعتر النامي في التربة الرملية.

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أجري هذا البحث بمشتل كلية الزراعة بجامعة المنصورة خلال الموسمين 2023/2022. بهدف دراسة تأثير حامض الهيوميك (HA) وفطر الميكروهيزا (AMF) علي الصفات الخضرية والزيت الطيار والمكونات الكيمائية لنبات الزعتر ومعرفة تأثير كل منهم منفرداً أو بصورة متداخلة. تم ري النباتات بحامض الهيوميك كمنشط حيوي شهرياً بتراكيز (0، 0.5، 1 و 2%) المزروعة في أصص تحتوي على تربة رملية مخلوطة ب الميكروهيزا أو بدون. أظهرت النتائج أن إضافة فطر الميكروهيزا للتربة أدى إلى زيادة معنوية في معظم الصفات المدروسة مثل النمو والمحصول ومحتوى النبات من الكلوروفيل والكربوهيدرات الكلية ونسبة الزيت العطري. أدى دمج فطر الميكروهيزا مع حمض الهيوميك بتركيز 1٪ إلى زيادة كبيرة في الوزن الطازج بنسبة تتراوح بين (16-46٪)، والكلوروفيل (84٪)، والكربوهيدرات (4-32٪)، وإنتاج الزيت (75-80٪). أدى إضافة حمض الهيوميك بتركيز 1% أثر أيضًا على مكونات الزيت. ونستنتج من النتائج أن فطر الميكروهيزا (AMF) وحمض الهيوميك يزيدان من نمو وإنتاج محصول الزيت في نبات الزعتر وزيادة قيمته التجارية.