

Response of Chemical Traits and Essential Oil Production of Thyme (*Thymus vulgaris* L.) to Mycorrhiza, Irrigation Water Quality, and Growing Medium

Nawar Nameer Hashim^{1*}, Thamer Abdullah Zahwan²

¹Department of Horticulture and Landscape Design, College of Agriculture, Tikrit University, Tikrit, Iraq.

²Department of Horticulture and Landscape Design, College of Agriculture, Tikrit University, Tikrit, Iraq.

*Corresponding author's email: nawar.n.hashim@st.tu.edu.iq

Email addresses of coauthors: thamir@tu.edu.iq

Abstract

Global agricultural sustainability is increasingly threatened by soil salinity, particularly for sensitive medicinal crops like thyme (*Thymus vulgaris* L.) in arid regions. This study evaluates the synergistic efficacy of bio-stimulation using Arbuscular Mycorrhizal Fungi (AMF) and organic soil amendments (compost) as an eco-friendly strategy to mitigate salinity stress. A field experiment was conducted in Tikrit-Iraq during the spring season of 2025 using a Randomized Complete Block Design (RCBD) with a factorial arrangement. The study investigated three factors: irrigation water quality (Fresh W0 vs. Saline W1), mycorrhizal inoculation (Non-inoculated M0 vs. Inoculated M1), and organic compost levels (0%, 10%, and 20%). Results demonstrated that saline irrigation independently caused significant reductions in vegetative traits and mineral uptake (N, P, K) due to osmotic and ionic stress, while inducing a defensive accumulation of carbohydrates (54.00%) in untreated stressed plants (W1M0O0). Conversely, the application of AMF and compost significantly alleviated these adverse effects. The tripartite interaction between saline water, mycorrhiza, and 20% compost (W1M1O2) proved to be the most critical finding; it successfully neutralized salinity stress, restoring leaf area to 1723.04 cm² and fresh weight to 1408.70 g—values statistically comparable to plants irrigated with fresh water. Furthermore, the optimal non-stressed combination (W0M1O2) maximized essential oil content to 4.47%, compared to only 1.97% in the stressed control. The study concludes that integrating mycorrhizal inoculation with organic composting provides a potent biological buffering system. This approach enhances nutrient availability and physiological resilience, offering a viable, sustainable protocol for cultivating high-quality medicinal thyme.

Keywords: Arbuscular Mycorrhiza; Compost; Medicinal plants; Salinity stress; *Thymus vulgaris*; Vegetative growth.

Introduction

Medicinal and aromatic plants are considered an integral part of the cultural and medical heritage of humanity [21], as they have been and continue to be the main source of raw materials used in the manufacture of drugs, cosmetics, and food products [12]. In light of the contemporary global trend towards returning to nature

and reducing reliance on synthetic chemical compounds, the economic importance of these plants has doubled [6].

Thyme (*Thymus vulgaris* L.) stands out as one of the most important plants belonging to the Lamiaceae family due to its multiple uses and its uniqueness in having a chemical content rich in volatile oils and phenolic compounds that possess

antioxidant and antimicrobial properties [9].

Recent studies by [20] indicate that the global demand for thyme oil has increased by 15% annually due to its medicinal properties as an anti-inflammatory and antioxidant.

However, the stability of this chemical content faces major physiological challenges due to the degradation of agricultural ecosystems, making the trend towards "Biostimulation" an indispensable necessity to ensure production sustainability and the quality of the active substance [4].

Arbuscular Mycorrhizal Fungi represent one of the finest models of biological symbiosis in nature. The function of these fungi is not limited to extending a network of hyphae that goes beyond the root depletion zone to reach phosphorus and water, but extends to modifying the gene expression of the host plant [2].

A study by [27] demonstrated that bio-fertilizers significantly improved Thyme quality through better macronutrient absorption (N, P, K increased by 16.9%, 19.5%, and 18.6%, respectively). The integrated mycorrhizal treatment maximized secondary metabolites, resulting in a 71% increase in essential oil content and a 42.7% rise in crude oil yield relative to the control.

[2] demonstrated that mycorrhizal inoculation enhances the productivity and quality of *Thymus satureioides* L. and *Thymus pallidus* L. even under non-stress conditions, The study reported significant biomass increases of 37.1% and 52.4%, respectively, alongside crude oil yield improvements of 21% and 74%, suggesting a correlation between morphological growth and photosynthetic efficiency. Furthermore, the fungal symbiosis significantly boosted nutrient acquisition, particularly Nitrogen rising to 0.9–1.0%) and Potassium reaching 88–105 mg/g, confirming the role of mycorrhiza in

optimizing plant nutrition and secondary metabolite profiles.

Irrigation water is considered the main determinant of plant growth in arid regions, However the challenge does not lie only in the quantity of water but in its quality. When using irrigation water with high salinity levels or well water, the thyme plant is exposed to double stress osmotic stress that prevents roots from withdrawing water, and ionic stress resulting from the accumulation of sodium and chloride ions, as shown by a study by [3].

[26] reported that salinity stress exerts a distinct negative impact on the growth of *Thymus vulgaris* L., primarily due to severe nutritional imbalances. The study highlighted a significant depletion in leaf mineral concentrations, with Potassium (K⁺) decreasing by 37–72% and Calcium (Ca²⁺) by 23–26% over four weeks, attributed to ionic competition with Sodium (Na⁺). Regarding essential oils, while salinity initially reduced key constituents like alpha-pinene and carvacrol, an age-dependent increase was observed in other bioactive compounds, where Thymol and Linalool levels rose by 15% and 17%, respectively, as plants matured.

[23] conducted a comprehensive analysis of the interactive effects of growth stage and soil water availability on *Thymus armeniacus* L. and *Thymus kotschyanus* L. Gas chromatography revealed distinct species-specific chemical profiles. The essential oil of *T. armeniacus* was dominated by gamma-Terpinene (16.65–23.58%), p-Cymene (6.70–16.56%), and alpha-Pinene (12.16–15.78%). In contrast, *T. kotschyanus* contained high levels of gamma-Terpinene (17.69–28.11%) and p-Cymene (17.78–41.90%), with a notable absence of alpha-Pinene. Furthermore, Thymol content varied significantly relative to the control, being highly abundant in *T. kotschyanus* (114.10–

157.30%) while remaining minimal in *T. armeniacus* (15.70–32.00%).

The addition of agricultural media such as compost goes beyond merely being a source of macronutrients (NPK) it acts as an environmental regulator for the soil, as it increases the soil's organic matter content, improves soil porosity, and raises the water-holding capacity, which is vital when using stressful irrigation water. Chemically, the humic and fulvic acids resulting from the decomposition of organic matter act as chelating agents that increase nutrient availability [19].

A study by [22] on Thyme revealed that organic compost amendments significantly outperformed the control, increasing essential oil content from 2.1% to 6.4%. The treatment also enhanced the chemical profile, with major compounds (Carvacrol, p-Cymene, gamma-Terpinene, and Thymol acetate) constituting over 60% of the oil. Specifically, compost application resulted in the highest concentrations of Carvacrol (36.8%) and gamma-Terpinene (18.7%).

[13] investigated the impact of various organic amendments on wild thyme. The study found that compost application significantly enhanced macronutrient absorption compared to the control, raising Nitrogen (N), Phosphorus (P), and Potassium (K) levels to 2.48%, 3.6%, and 14.8%, respectively.

Materials and Methods

The experiment was conducted in the Department of Horticulture and Landscape Design / College of Agriculture

/ Tikrit University at the geographical location (34.68044, 43.6485) during the spring growing season of 2025 on medicinal thyme plants.

The purpose was to study the role of Mycorrhiza fungi and the addition of media (organic compost) under the influence of two types of irrigation water (fresh and saline) to evaluate a set of traits, including the plant's content of mineral elements and carbohydrates, in addition to the crude oil percentage.

A plot of land was selected to implement the experiments, and the field was divided into three blocks (replicates). Each block included twelve treatments (experimental units) resulting from the interactions of the study factors, Each treatment within the block contained 3 plants planted in cylindrical pots with dimensions (50 cm diameter * 50 cm height) and a volume of 100 liters. A distance of 50 cm was left between treatments and 1 meter between one replicate and another. A drip irrigation system was installed with 6 lines for each block.

Loamy soil transported from the river basin was used in the pots samples were taken from it at different heights to determine its chemical and physical characteristics and properties, which were analyzed in the laboratories of the Department of Soil and Water / College of Agriculture, Tikrit University, and its properties are shown in Table 1.

Table 1: Chemical and Physical Properties of Field Soil

No	Parameter	Unit	Value
1	Nitrogen (N)	mg/kg (ppm)	200
2	Potassium (K)	mg/kg (ppm)	150
3	Phosphorus (P)	mg/kg (ppm)	10
4	Electrical Conductivity (EC) / Salinity	mmhos/cm (dS/m)	0.5
5	pH (Acidity)	--	6.8
6	Gypsum	%	2
7	Lime (CaCO ₃)	%	0.5
8	Organic Matter	%	1.5
9	Sand	%	40
10	Silt	%	35
11	Clay	%	25
12	Soil Texture	Class	Loam

The study factors were as follows:

1. Addition of Mycorrhiza Fungi (M):

Used at two levels:

- **M0:** Without Mycorrhiza addition.
- **M1:** Addition of Mycorrhiza fungus (*Glomus Power 200Gm Mycorr-hizal Biofertilizer*), produced by the Indian company Orjit Biotech, at planting 3 g.plant⁻¹ by dissolving it in 2.5 liters of water according to the manufacturer's recommendations. The suspension was added by spraying directly onto the soil after planting the plant in the pots, and the seedlings were irrigated with it in the evening after planting.

2. Irrigation Water Type (W):

Two types of irrigation water were used:

- **W0:** Fresh water with low salinity, average electrical conductivity (0.5 dS/m).
- **W1:** Well water (wells of the College of Agriculture Research Station / Horticulture Department) with relatively high salinity, average electrical conductivity (4.6 dS/m).

3. Agricultural Medium (Compost) (O):

Compost was used at three levels:

- **O0:** Without compost addition.
- **O1:** Addition of compost at a rate of 10% of the soil weight in the pot.
- **O2:** Addition of compost at a rate of 20% of the soil weight in the pot.

Table 2: Physical and Chemical Properties of the Compost Used

.No	Parameter	Unit	Value
1	Moisture Content	%	15
2	Organic Matter Content	%	45
3	pH Level	--	6.7
4	C:N Ratio	Ratio	1:10
5	Salinity (EC)	mS/cm	0.25
6	Diseases & Pests	--	Free from insect pests, diseases, and nematodes
7	Particle Size & Form	--	Friable, homogeneous, non-caking, granular
8	Odor	--	Odorless
9	Total Nitrogen (N)	mg/kg (Dry Matter)	(%2.4~) 24,380
10	Phosphorus (P ₂ O ₅)	mg/kg (Dry Matter)	(%0.9~) 9,250
11	Potassium (K ₂ O)	mg/kg (Dry Matter)	(%2.25~) 22,500

Studied Traits:

1:-Nitrogen Percentage in Leaves (%): The plant leaves' content of nitrogen was estimated using the Micro-Kjeldahl method [17].

2:-Phosphorus Percentage in Leaves (%): The leaf content of phosphorus was estimated using ammonium molybdate and ascorbic acid, and then measured using a Spectrophotometer at a wavelength of 882 nm [17].

3:-Potassium Percentage in Leaves (%): The content of thyme plant leaves of potassium was estimated relying on the method using a Flame photometer device [17].

4:-Percentage of Carbohydrate Content in Plant (%): Simple and complex carbohydrates are hydrolyzed in

the presence of concentrated sulfuric acid into monosaccharides that convert into furfural compounds, which react with phenol to form a yellow-orange color. The color intensity is measured at 490 nm, and the carbohydrate content is directly proportional to its quantity in the sample. Fresh thyme leaves are collected, washed with distilled water, and dried in an oven at 60–70°C until constant weight, then ground well to obtain a fine powder. Carbohydrates are extracted using 0.1 g of dry thyme powder, adding 10 ml of hot distilled water, then heating in a water bath at 80°C for 30 minutes. The solution is then cooled and filtered. The filtrate is used for analysis by drawing the standard curve. This is done by taking 1 ml of the sample extract, adding 1 ml of 5% phenol, and adding 5 ml of concentrated sulfuric acid with slight shaking. The tubes are left for 20 minutes, then absorbance is measured at 490 nm [8].

5:-Crude Oil Percentage (%): Thyme oil was extracted using the

Soxhlet apparatus, which operates on the principle of continuous extraction of compounds with low solubility in an organic solvent (ethanol) capable of dissolving the essential oil. The sample was prepared by drying and grinding thyme leaves to increase the surface area, then placing them in a filter paper thimble. The thimble containing thyme leaves is placed in the main chamber of the Soxhlet apparatus, and the apparatus is mounted on top of a flask containing the solvent (ethanol), then a condenser is added at the top. The solvent is heated in the flask, evaporates, and rises to the top through a side tube. The solvent vapor passes through the condenser at the top, turns into liquid, and drips onto the sample in the extraction chamber. The extraction tube fills gradually with the solvent until it reaches a certain level, then a side siphon tube drains the solvent saturated with essential oil back into the main flask. This cycle is repeated several times, ensuring complete extraction of the compounds. After the extraction process ends, the essential oil is separated from the solvent using a rotary evaporator. This method is considered very effective in extracting oils, especially those with low solubility in the solvent used, ensuring a high concentration of oil at the end of the process. The oil percentage is calculated according to the following equation:[5]

$$\text{Crude Oil \%} = (\text{Crude Oil Volume ml} / \text{Sample Weight g}) \times 100 .$$

Results and Discussion

1. Effect of Mycorrhiza, Irrigation Water Type, and Compost on Nitrogen Percentage in Leaves

The data presented in the table indicate that level W0 significantly excelled by recording an average of 2.31%

compared to level W1, which recorded a decrease at 1.77%. because of the high concentrations of specific ions in W1 such as Cl⁻ may have competed with nitrogen ions specifically NO₃⁻ for uptake sites on root membranes, leading to a nutrient imbalance [11].

In the same direction, the second factor showed a clear positive effect at level M1, which excelled with an average of 2.37% over level M0, The fungal hyphae extend far beyond the depletion zone of the root hairs, effectively increasing the absorptive surface area of the root system This allows the plant to access soil nitrogen resources that are physically unavailable to non-inoculated roots [25].

As for the third factor O, it showed a regular ascending behavior where the nitrogen percentage increased directly with the increase in addition levels, with level O2 giving the highest general average of 2.43% , significantly outperforming levels O1 and O0 respectively. Increasing the level of compost increases the total pool of organic nitrogen in the soil Through the process of mineralization, soil micro-organisms degrade this organic matter, releasing inorganic nitrogen forms NH₄⁺ and NO₃⁻ that are available for plant absorption [14].

The triple interaction between the best levels of individual factors (W0M1O2) led to the highest value in the experiment, reaching 3.20%, compared to W1M0O0 which recorded the lowest nitrogen content of 1.12% , combining AMF with organic amendments like compost creates a synergistic effect that significantly enhances soil nutrient availability and plant uptake compared to individual applications [7].

Table 1:Effect of Mycorrhiza, Irrigation Water Type, and Compost on Nitrogen Percentage in Leaves

Interaction / Treatment	O0	O1	O2	Interaction Mean (W×M)
Three-way Interaction (W×M×O)				
W0M0	H 1.53	E 2.07	C 2.25	C 1.95
W0M1	D 1.89	A 2.89	A 3.20	A 2.66
W1M0	I 1.12	H 1.53	G 1.73	D 1.46
W1M1	H 1.45	D 2.23	B 2.53	B 2.07
General Mean (O)	C 1.50	B 2.18	A 2.43	--
Two-way Interaction (W×O)				General Mean (W)
W0	D 1.71	B 2.48	A 2.73	A 2.31
W1	E 1.29	C 1.88	B 2.13	B 1.77
Two-way Interaction (M×O)				General Mean (M)
M0	E 1.33	C 1.80	B 1.99	B 1.71
M1	D 1.67	B 2.57	A 2.87	A 2.37

2. Effect of Mycorrhiza, Irrigation Water Type, and Compost on Phosphorus Percentage in Leaves

The results of the general means show clear significant differences for the effect of the studied factors. Level W0 of the first factor excelled by recording a general average of 0.61 compared to level W1 which recorded 0.48, and level M1 of the second factor excelled with an average of 0.60 over level M0.

Also, the third factor O excelled significantly, as level O2 achieved the highest general average of 0.71, outperforming levels O1 and O0 sequentially . This superiority of individual levels was clearly reflected in the outcome of the triple interaction (W/M/O), where the interaction treatment

W0M1O2 recorded the highest value for the studied trait in the experiment, reaching 0.87, indicating a high response between these three levels. In contrast, combining the levels with low effect in the combination W1M0O0 led to recording the lowest average of 0.33. Accordingly, the treatment W0M1O2 is considered the most efficient for improving this trait , This is attributed to the myco-rrhizae activating a defense mechanism known as local acidification, The fungus secretes hydrogen protons (H⁺) into the rhizosphere immediately surrounding the hyphae, locally lowering the pH. This localized drop in pH is sufficient to solubilize calcium phosphate compounds precipitated by salinity, thereby releasing and sequestering phosphorus. This process ensures a steady supply of phosphorus for the plant, which is essential for mitigating salt stress [15].

Table 2: Effect of Mycorrhiza, Irrigation Water Type, and Compost on Phosphorus Percentage in Leaves

Interaction / Treatment	O0	O1	O2	Interaction Mean (W×M)
Three-way Interaction (W×M×O)				
W0M0	HG 0.43	EF 0.53	B 0.73	0.56
W0M1	FG 0.50	CD 0.61	A 0.87	0.66
W1M0	I 0.33	H 0.41	E 0.54	0.42
W1M1	G 0.46	F 0.49	C 0.67	0.54
General Mean (O)	C 0.43	B 0.51	A 0.71	--
Two-way Interaction (W×O)				General Mean (W)
W0	C 0.46	B 0.57	A 0.80	A 0.61
W1	D 0.39	C 0.45	B 0.61	B 0.48
Two-way Interaction (M×O)				General Mean (M)
M0	E 0.38	C 0.47	B 0.64	B 0.49
M1	C 0.48	B 0.55	A 0.78	A 0.60

3. Effect of Mycorrhiza, Irrigation Water Type, and Compost on Potassium Percentage in Leaves

The table data indicate the presence of clear significant effects for the individual treatments and their interactions. The general means showed the superiority of level W0 by recording 2.54 significantly over level W1, and the superiority of level M1 with an average of 2.56 over its counterpart M0. Meanwhile, the third factor recorded an increase with the increase in concentration, as level O2 achieved the highest general average of 2.76, significantly outperforming the other two levels. These results were reinforced when studying the triple interaction, as the compatibility between the best studied levels in the interaction W0M1O2 led to maximizing the trait value to record the highest rate in the experiment reaching

3.10 compared to the rest of the interactions. While the absence of improvement factors in treatment W1M0O0 led to recording the lowest average of 1.77, confirming the high efficacy of the optimal triple combination in improving the indicators of the studied trait, This is attributed to changes in the Cation Exchange Capacity (CEC) and the presence of humus derived from organic matter decomposition. The high density of negative charges on the humic surfaces enables the retention of positive potassium cations (K^+) through electrostatic attraction, preventing them from leaching with irrigation water. Due to this interaction, potassium is released slowly to the roots as needed, ensuring a sustainable supply throughout the growing season. This mechanism likely explains the superiority of the interaction treatment (W0M1O2), which reached 3.10% [18].

Table 3:Effect of Mycorrhiza, Irrigation Water Type, and Compost on Potassium Percentage in Leaves

Interaction / Treatment	O0	O1	O2	Interaction Mean (W×M)
Three-way Interaction (W×M×O)				
W0M0	F 2.03	ED 2.37	B 2.83	B 2.41
W0M1	D 2.29	C 2.65	A 3.10	A 2.68
W1M0	G 1.77	F 2.03	E 2.27	D 2.02
W1M1	F 2.04	D 2.41	B 2.85	B 2.43
General Mean (O)	C 2.03	B 2.36	A 2.76	--
Two-way Interaction (W×O)				General Mean (W)
W0	C 2.16	B 2.51	A 2.96	A 2.54
W1	D 1.90	C 2.22	B 2.56	B 2.23
Two-way Interaction (M×O)				General Mean (M)
M0	E 1.90	C 2.20	B 2.55	B 2.21
M1	C 2.17	B 2.53	A 2.98	A 2.56

4. Effect of Mycorrhiza, Irrigation Water Type, and Compost on Carbohydrate Percentage

The table data illustrate noticeable significant variations between the experiment treatments. The general means showed the superiority of level W1 by recording 41.50 compared to level W0, and the superiority of level M0 with an average of 42.44 over its counterpart M1. Unlike the previous tables, factor O showed a descending effect where level O0 (no addition) achieved the highest general average of 46.17, and values decreased significantly with increased levels in O1 and O2. Consistent with the effect of individual factors, the triple interaction produced a clear superiority in the treatment W1M0O0, which recorded the highest value for the studied trait reaching 54.00, occupying the first statistical rank (A). In contrast, combining the levels that recorded the lowest averages in the combination W0M1O2 led to a decrease in the trait value to its lowest

level in the experiment at 20.67. Due to the symbiotic carbon cost and source-sink dynamics, mycorrhizal fungi as heterotrophic organisms rely entirely on the host plant for carbon and nutrients. Studies indicate that the fungus consumes between 10% and 20% of the plant's photosynthates. In the M1 treatment, a significant portion of carbohydrates was translocated from the leaves (source) toward the roots (sink) to sustain the extensive fungal network. This reduction in leaf carbohydrates serves as evidence of an accelerated metabolic turnover rate. As previously demonstrated, inoculated plants exhibit rapid growth, requiring the oxidation of sugars through cellular respiration to generate the energy (ATP) necessary for cell division. Furthermore, carbon skeletons from sugars

are diverted to synthesize cellulose cell walls and proteins, thereby preventing sugar accumulation in the leaves [24].

Table 4:Effect of Mycorrhiza, Irrigation Water Type, and Compost on Carbohydrate Percentage

Interaction / Treatment	O0	O1	O2	Interaction Mean (W×M)
Three-way Interaction (W×M×O)				
W0M0	CD 42.33	E 34.67	F 30.67	B 35.89
W0M1	D 40.67	G 21.67	G 20.67	C 27.67
W1M0	A 54.00	AB 49.33	BC 43.67	A 49.00
W1M1	B 47.67	F 29.00	F 25.33	B 34.00
General Mean (O)	A 46.17	B 33.67	C 30.11	--
Two-way Interaction (W×O)				General Mean (W)
W0	B 41.50	D 28.17	E 25.67	B 31.78
W1	A 50.83	B 39.17	C 34.50	A 41.50
Two-way Interaction (M×O)				General Mean (M)
M0	A 48.17	B 42.00	C 37.17	A 42.44
M1	B 44.17	D 25.33	D 23.00	B 30.83

5. Effect of Mycorrhiza, Irrigation Water Type, and Compost on Crude Oil Percentage of Thyme Plant

The data presented in the table indicate the presence of clear significant effects for the studied treatments. Level W0 in the first factor excelled, recording a general average of 3.40 compared to level W1. The second factor also showed superiority for level M1 with an average of 3.59 over its counterpart M0. Regarding the third factor O, a significant increase in averages was observed with the increase in level, where O2 achieved the highest general average of 3.80, differing significantly from the other levels. This positive trend culminated in the interaction, where the combination that brought together the best levels W0M1O2 led to recording the highest value for the

trait in the experiment reaching 4.47, occupying the first statistical rank. Conversely, combining the least effective levels in the combination W1M0O0 led to a decrease in the average to the lowest value reaching 1.97.

The mycorrhizal fungi transmit chemical signals to the host plant indicating the presence of salinity and osmotic stress. This triggers the plant to allocate resources toward the synthesis of crude oils not merely as secondary metabolites, but as potent antioxidants and membrane-protective agents. Due to its lipophilic nature, the crude oil composition may contribute to the stabilization of cellular membranes under salt stress. This explains the maintenance of high crude oil content in inoculated plants despite saline conditions [16].

Table 5: Effect of Mycorrhiza, Irrigation Water Type, and Compost on Crude Oil Percentage of Thyme Plant

Interaction / Treatment	O0	O1	O2	Interaction Mean (W×M)
Three-way Interaction (W×M×O)				
W0M0	hi 2.48	f 3.07	d 3.52	c 3.02
W0M1	g 2.80	c 4.07	a 4.47	a 3.78
W1M0	j 1.97	i 2.38	f 2.95	d 2.43
W1M1	h 2.58	e 3.37	b 4.25	b 3.40
General Mean (O)	c 2.46	b 3.22	a 3.80	--
Two-way Interaction (W×O)				General Mean (W)
W0	d 2.64	b 3.57	a 3.99	A 3.40
W1	e 2.28	c 2.88	b 3.60	B 2.92
Two-way Interaction (M×O)				General Mean (M)
M0	e 2.23	d 2.73	c 3.23	B 2.73
M1	d 2.69	b 3.72	a 4.36	A 3.59

Conclusions

Most traits (four out of five) followed a similar and unified direction in response to treatments. In the vast majority of results, level W0 excelled over W1 and level M1 over M0, while factor O had a clear direct effect as values increased with increasing levels to reach their peak at O2. This compatibility was reflected in the triple interaction where the combination W0M1O2 was the most efficient and appropriate to maximize the values of these traits such as Nitrogen, Phosphorus, Potassium content, and crude Oil content, achieving the highest averages with a

In contrast to this general trend, one trait (Table 4/Carbohydrates) deviated in its behavior, showing a completely opposite response where level W1 and M0 excelled, and the best effect was for the zero level O0, making the combination W1M0O0 the highest for this specific trait. In general, the results indicate that the fertilizer combination or composite treatment W0M1O2 is the optimal choice for improving most of the vital and qualitative indicators studied in the experiment, except for the carbohydrate

significant difference from the rest of the interactions, In this symbiotic relationship, the plant relies on fungal hyphae for nutrient acquisition rather than depleting its energy reserves (carbohydrates) to develop an extensive lateral root system. This explains the observed reduction in root branching in inoculated plants, contrasted by an increase in absorptive efficiency and overall productivity. By outsourcing nutrient uptake to the fungal network, the plant conserves metabolic energy, redirecting it toward vegetative and floral development [1].

percentage trait which showed a different behavior requiring opposite conditions (W1M0O0) to increase it. This indicates that the plant produced sugars but they were not used due to the lack of new growth to consume them, so they accumulated in the leaves and turned into starch additionally the plant resorts to accumulating soluble sugars in vacuolar sap to lower the cell's water potential which helps it withdraw water from saline soil and prevent dehydration [10].

References

[1]

- Ahmadvand, M., Javanmard, A., Haghaninia, M., & Morshedloo, M. R. (2022). Effects of Myco-Root biofertilizer application on quantity and quality of *Thymus vulgaris* L. essential oil in intercropping with *Cicer arietinum* L.
- [2] Akachoud, O., Bouamama, H., Facon, N., Laruelle, F., Zoubi, B., Benkebboura, A., ... & Lounès-Hadj Sahraoui, A. 2022. Mycorrhizal inoculation improves the quality and productivity of essential oil distilled from three aromatic and medicinal plants: *Thymus satureioides*, *Thymus pallidus*, and *Lavandula dentata*. *Agronomy*, 129, 2223.
- [3] Al-falah, M., Chtouki, M., Bouharroud , R., Boukhari, M. E. M. E., Ennoury, A., Beniaich, A., ... & Lyamlouli, K. 2025. Irrigation and phosphorus-driven adaptations in rosemary and thyme: a comparative morphophysiological and biochemical assessment. *BMC Plant Biology*.
- [4] Baltazar, M., Correia, S., Guinan, K. J., Sujeeth, N., Bragança, R., & Gonçalves, B. (2021). Recent advances in the molecular effects of biostimulants in plants: An overview. *Biomolecules* , 11 (8), 1096.
- [5] Canigüeral, S. 2025. The contribution of the European Pharmacopoeia to the quality control of herbal medicinal products Council of Europe. Section 2.8.12.
- [6] Ekor, M. (2014). The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology*,4,177. <https://doi.org/10.3389/fphar.2013.00177>
- [7] El Yeznasni, H., Chafai, W., Bouchentouf, H., Smouni, A., & Khalid, A. (2025). First detection of Arbuscular mycorrhizal fungi (AMF) in the root system of plants growing under metallic trace elements stress in eastern Morocco at a polluted mining site and identification of spores in the soil. *Aust J Crop Sci.* 19(11):1150-1157 (2025) | ISSN:1835
- [8] Ismail, B. P. Ed.. 2024. *Nielsen's Food Analysis Laboratory Manual*. Springer.
- [9] Jalil, B., Pischel, I., Feistel, B., Suarez, C., Blainski, A., Spreemann, R., Roth-Ehrang, R.,&Heinrich, M. 2024. Wild thyme *Thymus serpyllum* L.: A review of the current evidence of nutritional and preventive health benefits. *Frontiers in Nutrition*, 11, 1380962. <https://doi.org/10.3389/fnut.2024.1380962>
- [10] Jia, P., Huang, Y., Wang, X., Zhu, Z., Dou, Y., Huang, Q., ... & Wang, A. (2025). Mechanisms of chemical polymers and microbial residues in affecting organic matter stabilization under varying carbon/nitrogen ratios during composting. *Chemical Engineering Journal*, 162879.
- [11] López-Bascón, M. A., & De Castro, M. L. (2020). Soxhlet extraction. In *Liquid-phase extraction* (pp. 327-354). Elsevier.
- [12] Newman, D. J., & Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products*, 83(3), 770–803. <https://doi.org/10.1021/acs.jnatprod.9b01285>
- [13]Rahimi, A., Gitari, H. I., Lyons, G., Heydarzadeh, S., Tunçtürk, M., & Tunçtürk, R. (2023). Effects of vermicompost, compost and animal manure on vegetative growth, physiological and antioxidant activity characteristics of *Thymus vulgaris* L. under water stress.

Yüzüncü Yıl Üniversitesi Tarım Bilimleri Dergisi, 33(1), 40–53.
<https://doi.org/10.29133/yyutbd.1124458>

- [14] Rui, W., Mao, Z., & Li, Z. (2022). The roles of phosphorus and nitrogen nutrient transporters in the arbuscular mycorrhizal symbiosis. *International Journal of Molecular Sciences*, 23(19), 11027. <https://doi.org/10.3390/ijms231911027>
- [15] Sheikhpour, S., Tohidi-Nejad, E., & Ghanbari, J. (2024). Investigating the effect of mycorrhiza on growth, phosphorus nutrition, and biomass production in *Thymus vulgaris* and *Zataria multiflora* under drought stress conditions. *Irrigation and Water Engineering*, 14(3), 262-281.
- [16] Sinha, A., & Kumar, S. (2026). Symbiotic Relationships Between Arbuscular Mycorrhizal Fungi and Essential Oil- Producing Herbs: A Review of Recent Advances. *Journal of Basic Microbiology*, 66(1), e70127.
- [17] Tang, L. 2024. Soil fertility, plant nutrition and nutrient management . *Plants*, 141, 34.
- [18] Vaiciulyte, V., & Ložienė, K. (2024). INFLUENCE OF HUMUS FERTILIZATION ON YIELD AND ESSENTIAL OIL ACCUMULATION OF THYMUS× CITRIODORUS. PROFESSIONAL STUDIES: Theory And Practice, 28(2), 54-63.
- [19] Wenneck, G. S., Saath, R., Moro, A. L., Carvalho, G. P. D. S., Santi, D. C., & Rezende, R. 2023. Physiological responses of *thymus vulgaris* and *oregano vulgaris* under different water management and application of fermented bokashi compost. *Acta Scientiarum. Agronomy*, 45, e60807.
- [20] Wirtu, S. F., Ramaswamy, K., Maitra, R., Chopra, S., Mishra, A. K., & Jule, L. T. 2024. Isolation, characterization and antimicrobial activity study of *Thymus vulgaris* . *Scientific Reports*, 141, 21573.
- [21] World Health Organization. (2013). *WHO traditional medicine strategy: 2014-2023*. World Health Organization. <https://apps.who.int/iris/handle/10665/92455>
- [22] Yadegari, M. (2024). The effect of organic and chemical fertilizers on quantitative and qualitative characters of essential oil in Thyme (*Thymus vulgaris* L.). *Eco-phytochemical Journal of Medicinal Plants*, 12(1), 1–17.
- [23] Yousefzadeh, K., Houshmand, S., Shiran, B., Mousavi-Fard, S., Zeinali, H., Nikoloudakis, N., ... & Fanourakis, D. (2022). Joint effects of developmental stage and water deficit on essential oil traits (content, yield, composition) and related gene expression: A case study in two *Thymus* species. *Agronomy*, 12(5), 1008.
- [24] Zhang, E., Wang, Y., Crowther, T. W., Sun, W., Chen, S., Zhou, D., ... & Yu, G. (2025). Mycorrhiza increases plant diversity and soil carbon storage in grasslands. *Proceedings of the National Academy of Sciences*, 122(7), e2412556122.2707//doi.org/10.21475/ajcs.25.19.11.p369.
- [25] Zou, Y. N., Wu, Q. S., & Kuča, K. (2021). Unravelling the role of arbuscular mycorrhizal fungi in mitigating the oxidative burst of plants under drought stress. *Plant Biology*, 23, 50–57.
- [26] Zrig, A., Tounekti, T., Abd Elgawad, H., Hamouda, F., & Khemira, H. (2020). Influence of light intensity and salinity on growth and antioxidant machinery of *Thymus vulgaris* L. *Indian journal of experimental biology.-New Delhi*, 58(5), 323-335.
- [27] Machiani, M. A., Javanmard, A., Ostadi, A., & Morshedloo, M. R. (2021).

Evaluation of essential oil yield and ecological indices in the intercropping of thyme (*Thymus vulgaris* L.) and soybean

(*Glycine max* L.) with application of arbuscular mycorrhizal fungus.