***Funneliformis Mosseae* Application Under Water Deficit Stress Improves The Drug Yield And Phytochemical Characteristics of Thyme (*Thymus vulgaris* L.) in Intercropping With Soybean (*Glycine Max* L.)**

Mostafa Amani Machiani

University of Maragheh

Abdollah Javanmard (✉ a.javanmard@maragheh.ac.ir)

University of Maragheh

Mohammad Reza Morshedloo

University of Maragheh

Ahmad Aghaee

University of Maragheh

Filippo Maggi

University of Camerino

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Abstract

Today, there is a considerable demand in the global herbal market for thyme (*Thymus vulgaris* L.) and its related products such as extracts and essential oil (EO). In order to comply with this objective, an improvement of its cultivation area under water scarcity conditions is required. On this basis, a 2-year field experiment was performed with 18 treatments and three replications. Three irrigation levels, including i) irrigation after depletion of 20% (I_{20}), 50% (I_{50}) and 80% (I_{80}) available water were applied as the first factor. The second factor was different cropping patterns including thyme sole culture (T_s), soybean–thyme intercropping (in proportion of 50:50 and 66:34) and the third factor was non-usage (control) and usage of arbuscular mycorrhizal fungi (AMF) as bio-fertilizer. According to our results, the thyme dry yield under moderate (I_{50}) and severe water deficit stress (I_{80}) decreased by 35 and 44% in the first year, and by 27 and 40% in the second year compared with non-stressed (I_{20}) plants, respectively. Also, the macro- and micro-nutrients of thyme leaves increased significantly in intercropping patterns after application of AMF. The maximum EO percentage of thyme was achieved in 50:50 intercropping ratio and moderate water deficit (I_{50}). The major constituents of thyme EO were thymol, γ -terpinene, *p*-cymene, camphene, 1,8-cineole, camphor, (*E*)-caryophyllene, carvacrol and myrcene. Interestingly, as the water deficit stress was intensified the content of thymol, γ -terpinene and *p*-cymene increased significantly. Generally, AMF application in intercropping ratio of 50:50 (soybean: thyme) may be proposed to farmers as an eco-friendly approach to achieve desirable EO quality and quantity in thyme under water deficit stress conditions.

Introduction

Conventional agricultural systems are based on cultivation and productivity using sole plant cultures with intensive application of chemical inputs including synthetic fertilizer, pesticides, herbicides and others¹. The expansion of intensive crop production systems for long terms caused several problems, such as the reduction of biodiversity and the environmental contamination. The increasing concern regarding the negative impacts of these conventional agricultural methods on the ecosystems suggests the exploration of alternative methods endowed with improved efficiency and sustainability as well as high productivity².

The intercropping system is one of the well-known sustainable and eco-friendly agricultural methods in which two or more plants are grown simultaneously. This provides interconnections between plants and leads to a more efficient use of nutrients, water and land and to an increase of the plant production and a reduction of damages by weeds, pests and plant diseases^{3,4}. Among the various intercropping systems available, intercropping based on legume species provides an added value in order to minimizing the adverse impact of nutrient (nitrogen transfer from legume to companion crops or N as an input for subsequent crops) and moisture in addition to improving system productivity and soil health^{5,6}.

The climate-changes around the world indicate clearly that annual maximum temperature and followed by the aridity is increasing⁷. Water deficit stress, known as one of the limiting abiotic factors, affects every aspect of the plant growth, morphology, physiology and productivity in the arid and semi-arid regions^{8,9}. In addition, the nutrient use efficiency (NUE) of chemical fertilizer is reduced under drought conditions due to the decrease of mobility and low rate of mineral diffusion. Thus, the lower NUE along with the negative effects given by the chemical fertilizer application (e.g., soil, air and water pollution, soil acidification, mineral depletion, decrease of soil micro-organisms and harmful effects on human health) forced the agrochemical companies to replace the chemical inputs with eco-friendly fertilizers. Recently, the interaction among plant and soil microbial population is a conventional method to limit the demand of synthetic fertilizers over sustainable agricultural systems. Arbuscular mycorrhizal fungi (AMF) are soil borne micro-organisms forming a mutualistic symbiotic association with more than 80% of plant species^{10,11}. The coexistence between AMF and host plants improves the conditions for an optimal plant growth. In medicinal and aromatic plants (MAPs), previous research indicated that inoculation of AMF in dill (*Anethum graveolens* L.), rose geranium (*Pelargonium graveolens* L.) and *Mentha x piperita* improves the nutrient uptakes and the essential oil (EO) productivity^{12,13}. MAPs are the reservoir of secondary metabolites (including EO and polyphenolic compounds) which are useful remedies for humans and also have a great importance in fine chemicals, pharmaceuticals, cosmetics, perfumery and food industry. Tohidi et al. (2019)¹⁴ noted that more than 80% of population in developing countries, especially Iran, use MAPs plant in their healthcare system. Thyme (*Thymus vulgaris* L.), belonging to Lamiaceae family, is a flowering perennial plant native to the Mediterranean regions, including North Africa, Southern Europe and Western Asia, and adaptable to a wide range of environmental conditions¹⁵. Thyme has a great economic importance worldwide, especially for the production of its EO which has been estimated in 50-100 ton/year. The EO extracted from thyme has been reported to be as one of the top 10 EOs for sale worldwide¹⁶. The aerial parts as well as the volatile constituents of thyme are used in the folk medicine to treat cough, diabetes, cold and chest infections due to the documented antiseptic, antibacterial, antifungal, antispasmodic, antitussive, expectorant and analgesic properties related mainly to the presence of the phenolic monoterpenes thymol and carvacrol^{17,18}. Besides these compounds, the thyme EO is also characterized by the presence of γ -terpinene and *p*-cymene which are recognized as the biogenetic precursors of thymol and carvacrol¹⁹.

Iran is basically an arid country, with an average annual rainfall ranging from 224 to 275 mm/year (70% less than global average)²⁰. Recently, a considerable demand for thyme and its different products has been registered around the world. In order to comply with this objective, the improvement of thyme cultivation area under water scarcity conditions, especially in the arid and semi-arid regions of the world such as Iran, is urgently needed. Thus, an experiment was aimed to compare the impacts of different water deficit levels and application of AMF on the morphological characteristics, nutrients uptake, EO content and EO yield of thyme in different intercropping systems with soybean.

Material And Methods

2.1. Area of study

The experiments were performed over two successful growing year (April–October 2018 and April–October 2019) at the Maragheh University research farm, East Azerbaijan Province, Iran. The field soil had a loam texture with pH of 7.53; the electrical conductivity (EC) and the TOC (total organic-carbon) content

was 1.216 dS/m and 0.76%, respectively. The contents of macro- and micro-elements and physico-chemical characteristics are listed in Table 1. Also, the monthly weather data in the research area are provided in Table 2.

2.2. Treatments

A split split plot experiment, based on a RCBD design with three replications and 18 treatments, was performed. The main factor was represented by different water deficit stress levels, including i) irrigation after depletion of 20% available water (I_{20}) as non-stressed, ii) 50% of available water (I_{50}) as moderate water deficit, and iii) 80% available water (I_{80}) as severe water deficit. The sub- factor was different cropping patterns, including i) thyme sole culture (T_s) and ii) soybean–thyme replacement intercropping series (in proportion of 50:50 and 66:34). The experimental treatments based on proportions of soybean–thyme intercrops pattern is given in Table 3. In addition, the third factor was represented by i) non-application (control) and ii) application of AMF as bio-fertilizer. Thyme and soybean were sown in 4 m long rows and the distance of each row was set to 0.4 m (Fig.1). Also, a 1.5 m distance was maintained between different treatments in order to exclude any influence of lateral water movement. For AMF treatments, the mycorrhizal fungal spores (*Funneliformis mosseae*, 1000 spore/10 g soil) were added to the soil at planting (Zist Fanavar Sabz Company, Iran). Based on the soil physico-chemical analysis, in all treatments, 60 kg/ha superphosphate triple and 60 kg/ha N (30 kg ha⁻¹ as starter, 30 kg ha⁻¹ in Vegetative growth) were added into the soil before planting and during planting, respectively. Thyme Seedlings were obtained from Pakan Bazr company, Isfahan, Iran and sown on 15 April 2018 with density of 20 plants per row and 20 cm row distance. Before sowing, the seeds of soybean cultivar Sari (obtained from Mazandaran Agricultural and Natural Resources Research) were inoculated with *Rhizobium japonicum* and sown on 5 April 2018 and 10 April 2019 in both growing years. The plant material and seeds were obtained under the supervision and permission of Maragheh University and according to national guidelines and all authors comply with all the local and national guidelines. In order to reduce the plant mortality, in the first month of growth the plants were watered normally. Water stress treatments were performed according to the maximum allowable depletion (MAD) percentage of the soil available water (SAW). The water stress treatments applied were 20, 50 and 80% MAD of SAW, including the non-stress (I_{20}), moderate water deficit (I_{50}) and severe water deficit (I_{80}), respectively. For measuring the soil water content, a Time-Domain Reflectometry (TDR) probe (Model TRIME-FM, England) was used. The readings were performed at the center of each experimental plot at a depth of 30 cm. The following equations was used for calculating the irrigation depth²¹:

$$SAW = (\theta_{fc} - \theta_{pwp}) \times d \times 100$$

$$I_d = SAW \times p$$

$$I_g = [I_d \times 100] / E_a$$

In these equations, SAW, θ_{fc} , θ_{pwp} , d, I_d , p, E_a and I_g are: soil available water, soil field capacity (27.4%), permanent wilting point (13.9%), soil layer depth (cm), irrigation depth (cm), fraction of SAW (20%, 50% and 80%) that can be depleted from the root zone, irrigation efficiency (%) averagely assumed 65%²² and the gross depth of irrigation (cm), respectively.

2.3. Measurements

2.3.1. Dry yield

At full flowering stage, the thyme aerial parts were harvested randomly on 15 August 2018 and 27 August 2019. The plants were randomly harvested from a 1.6 m² area (middle line of each treatment). After harvesting, the fresh weight of treatments was measured and shade dried to calculate the dry matter yield.

2.3.2. Mycorrhizal Fungal Colonization

To determine the root colonization percentage, the young fresh roots of thyme were collected from the depth of 10-20 cm of the soil. The root colonization was investigated according to the method described by Phillips and Hayman (1970)²³. Firstly, the roots were washed with water and then cut into the small pieces (1.5 cm length) and followed by in 10% potassium hydroxide at 90°C for 10 min. Afterwards, the segments were placed in 2% HCl at 25 °C for 10 min and then stained with trypan blue (0.05%) and lactic acid (80%) for 12 h. Eventually, the roots were washed and stored in lactic acid, glycerol and distilled water (lactic acid and glycerol in proportions of 1:1:1 v/v/v) solution. Finally, the colonization percentage of thyme root was measured according to the method of Giovannetti and Mosse (1980)²⁴.

2.3.3. Essential oil content and chemical analyses

40 g of dried aerial parts of herbs were roughly grounded and placed in a 1 L flask where 400 mL of distilled water and conventional hydro-distillation for 3 h, using a Clevenger-type apparatus, was performed. In order to remove possible water drops, Na₂SO₄ was added to each extracted EO and kept at 4°C in the refrigerator before analysis. For calculating the EO content and yield of thyme, the following equations were used:

$$EO \text{ content of thyme} = [\text{distilled EO (g)/ 40 g}] \times 100$$

$$EO \text{ yield of thyme (g/m}^2\text{)} = \text{dry yield of thyme (g/m}^2\text{)} \times EO \text{ content (\%)}$$

The thyme EO constituents were analyzed using GC–FID and GC–MS instruments GC-MS analysis was performed in an Agilent gas chromatograph (7990B) coupled to a 5977A mass spectrometer. The separation was done in a HP-5 MS capillary column (5% Phenyl Methylpolysiloxane, 30 m length, 0.25 mm i.d.,

0.25 μm film thickness). The following oven temperature was set up for the column: 10 min at 60°C, then the temperature raised with the rate of 3°C min⁻¹ to 250°C, finally held for 15 min at 250°C. The carrier gas (Helium) was used at a flow rate of 1 mL/min. Electron-impact (EI) was set to be 70 eV. The injector split ratio was 1:50 and mass range acquisition was from 40 to 400 m/z . Gas chromatographic (GC-FID) separations were performed in an Agilent technology instrument (Agilent 7990B, USA), coupled with a flame ionization detector (FID). VF-5MS column used in the GC-FID device had the same stationary phase and dimensions as the HP-5MS one. The oven temperature program was the same used for GC-MS analysis. Injector and detector temperatures were set at 230 and 240°C, respectively. Diluted essential oils (1: 100 in *n*-hexane) were used for the injection. The peak areas were normalized without using correction indices.

To identify EO constituents, a combinational procedure was performed including determination of arithmetic retention indices using coherence of homologous series of hydrocarbons (Supelco, Bellefonte, USA), comparing retention indices with those reported in the reference literature²⁵, and the computer matching of mass acquisition with the WILEY275 and NIST 05 libraries.

2.3.4. Plant Nutrient concentration

Thyme leaf samples were analyzed for nutrients concentrations. The Kjeldahl method was used to determine the N content. The concentration of K was also measured using flame photometry²⁶. P was determined by the yellow method²⁷. The content of P was determined at 470 nm using a spectrophotometer. An atomic absorption spectrophotometer (AA-6300 F; Shimadzu, Japan) was used to determine the concentration of micro-nutrients including Ca, Fe, Zn and Mg of each samples

2.4. Statistical analysis

The homoscedasticity and normality test of all data were checked with Levene test and Anderson-Darling methods, respectively. Analysis of variance (ANOVA) and the means comparison (compared with LSD at 5% probability level) were performed using the SAS software version 9.1.

Results

3.1. Root colonization percentage

In both years, the highest root colonization percentage was measured in the 50:50 intercropping ratio with non-stressed plants (I_{20}). Also, the lowest percentage of thyme root colonization (46.81% in the first year and 56.87% in the second year) was observed in the sole culture of thyme under severe stress (I_{80}) (Fig. 2).

3.2. Dry yield

The highest and lowest dry yield (in both years) was achieved in the sole culture of thyme with well-watered condition (I_{20}) and in the intercropping ratio of 66:34 with I_{80} water deficit (Fig. 3). Furthermore, in the first and second year, application of AMF enhanced the dry productivity by 17.3 and 14.5%, respectively, in comparison with control. However, the dry productivity in 50:50 and 66:44 intercrops decreased by 42 and 58% in the first year and by 38 and 59% in the second year, respectively, in comparison with thyme sole culture. Furthermore, the thyme dry yield in the moderate (I_{50}) and severe water deficit (I_{80}) decreased by 35 and 44% in the first year, and by 27 and 40% in the second year, respectively, in comparison with I_{20} (Table 4).

3.3. Nutrient concentrations

The most of macro- and micro nutrient concentrations was significantly impacted by single effects of water deficit levels, cropping patterns and AMF application. Also, interaction of water deficit levels $\times$ cropping patterns had a significant impact on Zn content (Table 5).

3.3.1. N concentration

In both years, the highest N concentration was calculated in 50:50 intercropping followed by 66:34. The lowest N concentration was recorded in the sole culture of thyme. The N content in 50:50 and 66:34 cropping patterns increased by 15 and 9% in the first year, and by 20 and 13% in the second year, respectively when compared with sole culture. Among three water deficit levels, the highest and lowest N content in both years was calculated in I_{20} and I_{80} , respectively. Moreover, the N content in the first and second year enhanced by 10.9 and 9.6% after application of AMF (Table 5).

3.3.2. P concentration

The P content of thyme leaves in the first and second year enhanced by 10.5 and 8.7% after application of AMF. Also, the highest and lowest P concentration was recorded in I_{20} and I_{80} water deficit levels, respectively. The P content in the I_{50} and I_{80} water scarcity conditions decreased by 17 and 30% in first year and by 18 and 29% in the second year, respectively, when compared with well-watered conditions (Table 5).

3.3.3. K concentration

The maximum K content (15.2 and 17.1 g/kg in 2018 and 2019, respectively) was recorded in the intercropping ratio of 50:50 and 66:34. The content of K decreased by enhancing the water deficit levels. The maximum and minimum concentration of K in both years was achieved in I_{20} and I_{80} , respectively. Overall, the K content in the first and second year increased by 14.1 and 13.2%, respectively, after application of AMF when compared with control (Table 5).

3.3.4. Ca concentration

The highest Ca content (4.4 and 5.3 g/kg in 2018 and 2019, respectively), was achieved in the intercropping ratio of 50:50. The concentration of Ca decreased under water deficit conditions. The maximum and minimum concentration of Ca in both years was achieved in I₂₀ and I₈₀ water deficits, respectively. The concentration of Ca in I₂₀ was 14 and 30% (in the first year) and 14 and 23% (in the second year) higher than those registered in I₅₀ and I₈₀ treatments, respectively. Overall, the Ca concentration in the first and second year enhanced by 11.3 and 5.8%, respectively, after application of AMF in comparison with control (Table 5).

3.3.5. Mg concentration

The maximum Mg content in thyme leaves (2.6 and 3.4 g/kg in 2018 and 2019, respectively) was recorded in the intercropping ratio of 66:34 and 50:50, corresponding to an increase by 9 and 7% in the first year, and by 17 and 14% in the second year in comparison with sole culture. Also, the content of Mg decreased under water deficit conditions. The maximum and minimum content of Mg in both years was achieved in I₂₀ and I₈₀ water deficit conditions, respectively. The concentration of Mg in the I₅₀ and I₈₀ water deficits decreased by 17 and 33% in the first year, and by 14 and 25% in the second year in comparison with well-watered conditions, respectively. Furthermore, the Mg concentration in the first and second year enhanced by 21.7 and 14.1% after application of AMF compared with control, respectively (Table 5).

3.3.6. Fe concentration

The highest Fe concentration of thyme leaves (377.8 and 503.8 mg/kg in 2018 and 2019, respectively) was recorded in the intercropping ratio of 50:50. The Fe content in the intercropping ratio of 50:50 and 66:34 increased by 22 and 6% in the first year and by 25 and 12% in the second year, respectively, when compared with the sole culture. The maximum and minimum concentrations of Fe in both years were achieved in the I₂₀ and I₈₀ water deficit conditions, respectively. The concentration of Fe in I₅₀ and I₈₀ decreased by 22 and 29% (in 2018), and by 18 and 28% (in 2019), respectively, in comparison with I₂₀ treatment. Overall, the Fe concentration in the first and second growing year increased by 18.1 and 14.6%, respectively, after application of AMF compared with control (Table 5).

3.3.7. Zn concentration

In first and second year, the highest Zn concentration was achieved in the thyme sole culture and 50:50 intercropping ratio with non-stressed water deficit (I₂₀), respectively. However, the lowest content of Zn in both years was recorded in thyme sole culture with severe water deficit (I₈₀) (Fig. 4). The Zn content in I₅₀ and I₈₀ water deficit conditions decreased by 11 and 24% (first year), and by 14 and 25% (second year) in comparison with I₂₀ treatment. Also, the Zn concentration increased by 6.3 (first year) and 8.1% with AMF inoculation (Table 5).

3.4. EO content

Between three levels of water deficit, the maximum EO productivity was measured in I₅₀ water scarcity that was 52 and 34% (in 2018), 22 and 4% (in 2019) higher than those observed in I₂₀ and I₈₀ treatments, respectively (Table 4). Based on the interaction of cropping patterns × AMF application, the highest EO content in both years was achieved in the intercropping ratio of 50:50 after AMF application. Also, the lowest EO content in both years was observed in the sole culture of thyme without usage of AMF (Fig. 5).

3.5. EO yield

In the first and second year, application of AMF increased the EO yield by 59.6 and 23%, respectively, in comparison with control (non-AMF). Based on the interaction of cropping patterns × water deficit, the highest EO yield in both years (5.04 and 9.46 g m⁻² in the first and second year) was achieved in the non-stress plants (I₂₀) in sole culture (Fig. 6). In addition, the EO yield in the intercropping ratio of 50:50 and 66:44 decreased by 17 and 53% in the first year, and by 19 and 55% in the second year, respectively, when compared with the sole culture. Finally, the EO yield in I₅₀ and I₈₀ water deficit stresses decreased by 3 and 35% in the first year, and by 9 and 31% in the second year, respectively, when compared with well-watered plants (I₂₀) (Table 4).

3.6. EO composition

In total, 29 compounds were identified in the thyme EOs, accounting for 97.87-99.74% and 97.06%-99.57% of the total compositions at the first and second year, respectively (Table 6 and 7).

In the first year, the main EO constituents were thymol (31.12-37.26%), γ -terpinene (13.14-17.48%), *p*-cymene (11.37-13.92%), camphene (6.52-8.52%), 1,8-cineole (2.94-5.83%), camphor (3.74-5.61%), (*E*)-caryophyllene (2.57-4.19%), carvacrol (2.01-3.41%) and β -myrcene (1.72-2.31%). The highest content of thymol was achieved in of 50:50 intercrop with moderate water deficit (I₅₀) and application of AMF. The content of thymol in the intercropping ratio of 50:50 and 66:34 increased by 6.3 and 5.9%, respectively, when compared with thyme sole culture. Also, the thymol percentage in the I₅₀ and I₈₀ water deficit increased by 7.7 and 3.5%, respectively, when compared with control (I₂₀). Furthermore, application of AMF increased the content of thymol by 6.3% in comparison with control. The highest content of γ -terpinene was observed in 50:50 intercrop under severe water deficit (I₈₀) and application of AMF. The content of γ -terpinene in the intercropping ratio of 50:50 and 66:34 increased by 14.6 and 4.4% when compared with thyme sole culture. Also, the γ -terpinene percentage in I₅₀ and I₈₀ water scarcity increased by 13.9 and 9.9% when compared with I₂₀. The highest percentage of *p*-cymene was measured in 66:34 intercropping with severe water deficit (I₈₀) and application of AMF. Also, the *p*-cymene percentage in I₈₀ water deficit increased by 4.8% when compared with non-stressed water deficit (I₂₀). Furthermore, application of AMF increased the content of *p*-cymene by 2.7% in comparison with control (Table 6).

In the second year, the main constituents of thyme EO were thymol (40.89-48.59%), γ -terpinene (16.93-21.55%), *p*-cymene (13.73-16.26%) and carvacrol (2.95-4.06%). Based on the results, the highest percentage of thymol was achieved in the intercropping ratio of 50:50 with moderate water deficit (I_{50}) and application of AMF. The content of thymol in the intercropping ratio of 50:50 and 66:34 enhanced by 4.2% compared with thyme sole culture. Also, the thymol percentage in I_{50} and I_{80} water stress conditions increased by 8 and 2.9%, respectively, in comparison with I_{20} . Overall, the application of AMF increased the content of thymol by 2.8% in comparison with control. The highest percentage of γ -terpinene was observed in 50:50 intercropping with severe water deficit (I_{80}) and application of AMF. The content of γ -terpinene in the intercropping ratio of 50:50 and 66:34 enhanced by 6.5 and 7.6%, respectively, when compared with thyme sole culture. Also, the γ -terpinene percentage in I_{50} and I_{80} water deficit conditions increased by 7.3 and 9.7%, respectively, when compared with I_{20} . Finally, the application of AMF increased the content of γ -terpinene by 9.3% when compared with control. The highest percentage of *p*-cymene was measured in 66:34 intercrop under severe water deficit (I_{80}) and application of AMF. The content of *p*-cymene in the intercropping ratio of 50:50 and 66:34 enhanced by 4.2 and 6.6%, respectively, when compared with thyme sole culture. Also, the *p*-cymene percentage in I_{80} water scarcity conditions increased by 5.3%, respectively, in comparison with I_{20} (Table 7).

Discussion

4.1. Root colonization

The results demonstrated that the root colonization percentage increased in intercropping patterns in comparison with sole culture. The higher AMF colonization in intercropping could be attributed by an improvement of the soil microbial communities through an enhancement of the organic matter and accessibility of legume-fixed nitrogen into the soil which have a positive effect on root colonization. Weisany et al. (2016)¹³ showed that the presence of legume species in intercropping patterns can increase the AMF diversity leading to an increase of root colonization in different intercropping patterns of dill/common bean. Similarly, Wahbi et al. (2016)²⁸ explained that the AMF hyphal density is higher in faba bean/wheat intercropping when compared with the respective sole cultures. In addition, the AMF root colonization of thyme decreased under higher water deficit levels which could be due to the reduction of soil microbial activities under drought conditions. Alike, Shukla et al. (2013)²⁹ concluded that the different soil conditions, especially moisture, changed the AMF root colonization. These authors noted that the maximum and minimum AMF colonization in the vigna mungo (*Vigna mungo* (L.) Hepper) and wheat (*Triticum aestivum* L.) was observed in the field capacity (FC) and half-field capacity (FC/2), respectively.

4.2. Nutrient concentrations

Our results showed that the macro- and micro-nutrients of thyme increased in intercropping patterns. The difference in rooting depth and root expansion of plants affects the competition of intercropping components over nutrients. Therefore, the higher nutrient content in intercrops could be attributed to the increase of the environmental use efficiency of intercropping components as a results of different root distribution (e.g., thyme is a shallow-rooted and soybean is a deep-rooted type crop) leading to the improvement of the nutrient uptakes at different depths of the soil³⁰. Duchene et al. (2017)⁴ showed that the nutrients availability in intercropping patterns improved due to the higher symbiotic nitrogen fixation by legumes, root exudation of enzymes such as phosphatases, carboxylates, and increase of soil acidity by production of H^+ in comparison with sole culture of plants.

Also, the nutrient concentrations of N, P, K, Ca, Mg, Zn and Fe improved after application of AMF compared with untreated plants. The extensive network of AMF hyphae in the plant roots increases the absorption rate and area leading to the enhancement of the nutrients availability and uptake of micro- and macro-elements, especially lowly mobile nutrients such as P^{10} .

Furthermore, the results of this study exhibited that nutrient concentration decreased by raising the water deficit levels. This could be explained by the reduction of nutrient supply through mineralization, decreasing mass flow and nutrient diffusion that affect the kinetics of nutrient uptake per unit of roots³¹. In addition, Bista et al. (2018)³² reported that the activity or concentration of major uptake-proteins for nutrients decreased under water stress and had a negative impact on the nutrient's uptake from roots. Also, these authors noted that the nutrients concentration is higher in the upper soil layers and drought stress reduced the nutrients absorption by decreasing the water uptake in the upper soil layers.

4.3. Yield

The results showed that dry matter yield of thyme significantly increased under sole culture. The decreasing of thyme dry matter in intercropping patterns could be attributed to lower plant density of thyme and competition for solar radiation, nutrition, space and other environmental resources³³. In addition, the shading of the companion plant (soybean) over thyme seedlings in intercropping patterns could decrease the photosynthetic rate, leading to a decrease in thyme productivity. Among intercropping patterns, thyme, in the ratio of 50:50, enjoyed optimal environmental resource conditions for their canopy, so that the availability and uptake of nutrients increased, leading to an increase in the yield.

Our results showed that the thyme yield decreased significantly with the increase of water deficit levels. Similar to other plants, thyme plants that tolerate water or drought stresses have apparently evolved mechanisms such as early flowering and structural traits including the reduction of plant leaf area and height and also metabolic responses, such as photosynthetic alterations and proline accumulations (data not shown), to survive under water stress conditions. Therefore, the observed decrease in the thyme growth parameters could be explained by the lower availability of sufficient moisture around the root zone and lower absorption of nutrients as a result of the lower proliferation of root biomass. These conditions had a negative impact on the chlorophyll pigments, the photosynthetic efficiency and finally plant yield²². Alike, Askary et al. (2018)¹⁷ showed that total dry matter yield in two different species of thyme (*T. daenensis* Celak. and *T. vulgaris*) decreased by 21.6 and 38.9% in the 67 and 33% FC (field capacity) in comparison with 100% FC, respectively

Also, our results demonstrated that application of AMF significantly increased the biomass yield of thyme. The mycorrhizal rhizosphere pH is lower due to the absorption of ammonium ions and releasing of H^+ ions. Reducing soil acidity and also exudation of organic acids and phosphatase enzymes increase the solubility and accessibility of nutrients to plant roots. Therefore, an improvement of plant growth parameters and productivity of thyme after AMF application could be due to the enhancement of the macro- and micronutrients uptake such as N, P, K, Ca, Fe, Zn and Mg through the expansion of the mycelium and the development of the root system. This increases the chlorophyll content affecting the photosynthetic rate and the photosynthate production. Moreover, a higher production of plant growth hormones such as gibberellin (affecting the longitudinal cellular growth and primarily stem internodes), auxin and cytokinin (affecting the cell division) has been observed^{10,44}. Golubkina et al. (2020)³⁵ showed that the AMF inoculation increased the biomass and dry yield of *Artemisia dracunculus* L., *Hyssopus officinalis* L. and *Lavandula angustifolia* Mill. compared with untreated plants.

4.4. Essential oil content

Although secondary metabolites such as those contained in MAPs essential oils are conventionally controlled by the plant genotypes, their biosynthesis is strongly affected by the environmental factors⁹. The results exhibited that the EO productivity of thyme increased in intercropping patterns. The nutrients accessibility, especially N, plays an important role in the EO gland cells size and EO productivity in MAPs³⁶. Therefore, the increment of EO content in planting patterns may be related to the higher accessibility of nutrients and atmospheric nitrogen fixation by legumes as well as the higher resource use efficiency of nutrients and water due to the superior complementarity (special, chemical, and temporal) in thyme/soybean intercropping patterns compared with pure cultures⁴.

Also, the thyme EO content increased after AMF application. The AMF colonization increases the EO productivity of MAPs by enhancing the plant accessibility to important nutrients such as N and P and other micronutrients including Fe and Zn. Copetta et al. (2006)³⁷ reported that AMF inoculation increased the EO productivity in basil (*Ocimum basilicum* L.) due to the increase of glandular trichomes in leaves. Also, these authors noted that the higher number of glandular trichomes after AMF colonization could be explained by the changes in plant hormones induced by AMF and also by an increase of nutrients and water uptakes with the expansion of the mycelium and development of roots.

In addition, the EO productivity of thyme increased in the moderate and severe water deficit conditions when compared with non-stressed plants. The increase of EO productivity under moderate and severe water stress could be explained by a decrease in the leaf area of plants leading to a raise of the glandular trichomes density and EO accumulation per unit of leaf tissue. Turtola et al. (2003)³⁸ noted that the increase of EO productivity in MAPs under drought stress conditions may be related to a low allocation of carbon to the growth, representing a trade-off between growth and plants defense. Govahi et al. (2015)²² concluded that the maximum EO content of sage (*Salvia officinalis* L.) was achieved in moderate drought stress. Also, the moderate and severe drought stress increased the EO productivity of sage by 109 and 84%, compared with non-stressed plants, respectively.

4.5. Essential oil yield

The EO yield of thyme increased in the sole culture and decreased after raising the water deficit stress levels. The EO yield, calculated from the herbage yield multiply with EO content and had a positive relationship with two mentioned factors. These differences in thyme EO yields could be due to the higher dry productivity in the sole culture and application of AMF and to the decrease of the dry productivity under water deficit conditions. In addition, the higher EO yield of thyme after application of AMF may be attributed to the higher dry matter and EO productivity. Likewise, Bahreinejad et al. (2013)²¹ showed that the EO yield in *T. daenensis* decreased under moderate and severe water stress due to a decrease of herbage yield in these conditions.

4.6. Essential oil composition

EOs are mixtures of low molecular weight compounds that are responsible for the characteristic aroma of MAPs. The ratio of one special compound or group of compounds to another affects the quality of EO in these herbs. The major constituents of thyme EO, namely thymol, γ -terpinene and *p*-cymene increased in the intercropping system and after application of AMF. The content of these constituents in MAPs depends on the availability of their precursors and energy source molecules such as acetyl-CoA, ATP and NADPH³⁹. The content of the mentioned compounds increased as a function of nutrients availability. Thus, the intercropping with soybean and the application of AMF enhanced the uptake of nutrients by plants, and the marker EO constituents increased. Likewise, Amani Machiani et al. (2019)⁴⁰ concluded that the EO constituents of *Foeniculum vulgare* Mill. and *Dracocephalum moldavica* L. improved in different intercropping patterns with *Phaseolus vulgaris* L. and after organic fertilizer usage (humic acid).

Among different water deficit levels applied in our experiments, the content of thymol, γ -terpinene and *p*-cymene increased in the moderate and severe water stress compared with well-watered conditions. This increase could be explained by the reduction of the plant growth under water scarcity conditions, and by the use of fixed carbon in the photosynthetic process for the production of secondary metabolites⁴¹. Tohidi et al. (2019)¹⁴ noted that the quality of thyme EO is strictly related to the content of thymol which is used for culinary, pharmaceutical and cosmetic purposes. Thus, in our experiments the quality of thyme EO constituents under moderate and severe water stress improved in intercroppings with AMF inoculation.

Conclusions

Water deficit or drought stress conditions (based on the severity and duration of the stress) inhibit the plant growth and reduce the quality and quantity of their derived products. Our study showed that the water deficit levels have a negative impact on dry matter yield of thyme. In contrast, the EO content of thyme increased under severe water deficit conditions (I_{50} and I_{80}), intercropping patterns and AMF-inoculated plants. The major EO constituents of thyme including thymol, γ -terpinene and *p*-cymene increased in intercropping patterns and with application of AMF. In addition, the micro- and macro-nutrients concentrations increased significantly in intercropping patterns and AMF-inoculated plants. Based on the obtained results, in the semi-arid and arid regions, in which water

deficit has a negative impact on the plant productivity, intercropping thyme with soybean under application of AMF as bio-fertilizer is recommended as a feasible and eco-friendly alternative strategy to improve the thyme EO quality and quantity compared with the sole culture.

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Tables

Table1. Physico-chemical properties of field soil (depth of 0-30 cm) an average over the two years.

Soil texture	Sand (%)	Silt (%)	Clay (%)	Field Capacity (%)	permanent wilting point (%)	Organic matter (%)	EC (ds.m ⁻¹)	pH	Amount of cation exchange capacity (Cmolc/kg)	Amount potassium (mg/kg)	Available phosphorus (mg/kg)	Total nitrogen (%)
sandy clay loam	47	24	29	27.4	13.9	0.76	1.216	7.53	25.8	516.3	12.6	0.09

Table 2. Monthly average temperature and total monthly precipitation of experimental site during two growing seasons and long-term averages.

Year	April	May	June	July	August	September	October
Monthly average temperature (°C)							
2018	12.6	16.6	24.1	30.2	27.7	23.6	15.9
2019	10.4	18.5	25.7	27.6	27.8	22.1	16.7
2-year mean	11.5	17.5	24.9	28.9	27.8	22.8	16.3
10-year mean	12.7	18.0	24.0	27.9	27.4	22.0	15.1
Total monthly precipitation (mm)							
2018	44.9	54.5	1.7	0.1	0	0.2	19.5
2019	51.3	37.8	4.2	0.0	0.0	0.0	6.3
2-year mean	48.1	46.2	3.0	0.1	0.0	0.1	12.9
10-year mean	39.4	16.6	3.4	0.4	0.3	1.7	17.1

Table 3. Experimental treatments based on proportions of soybean–thyme intercrops.

Soybean density (m ²)	Thyme density (m ²)	Proportion of thyme-soybean intercrops (%)	Proportion of thyme/soybean intercrops (plant m ²)
45	12.5	0-100 (sole culture)	12.5
45	12.5	50-50	23-6
45	12.5	66-34	30-4

Table 4. The dry matter yield, essential oil content and yield of thyme in different cropping patterns and irrigation levels with AMF application.

	Treatments	Dry yield (g m ⁻²)		Essential oil content (%)		Essential oil yield (g m ⁻²)	
		2018	2019	2018	2019	2018	2019
	T _s	419.3 a	533.7 a	1.13 c	1.78 c	4.7 a	9.4 a
Cropping Pattern	50:50	244.8 b	331.2 b	1.62 a	2.31 a	3.9 b	7.6 b
	66:34	175.1 c	219.5 c	1.32 b	1.95 b	2.2 c	4.2 c
	LSD	36.7	37.5	0.07	0.11	0.58	0.85
Fertilization	Control	257.52 b	337.1 b	1.28 b	1.95 b	3.07 b	6.35 b
	AMF	301.96 a	385.8 a	1.42 a	2.08 a	4.09 a	7.81 a
	LSD	29.99	30.64	0.06	0.09	0.47	0.69
	I ₂₀	379.01 a	465.9 a	1.11 c	1.78 b	4.11 a	8.17 a
Drought stress	I ₅₀	246.21 b	340.8 b	1.69 a	2.18 a	3.98 a	7.42 a
	I ₈₀	213.99 b	277.7 c	1.26 b	2.09 a	2.66 b	5.63 b
	LSD	36.73	37.53	0.07	0.11	0.58	0.85
Significance							
	C	**	**	**	**	**	**
	F	**	**	**	**	**	**
	D	**	**	**	**	**	**
	C × F	ns	ns	*	*	ns	ns
	C × D	*	**	ns	ns	**	**
	D × F	ns	ns	ns	ns	ns	ns
	C × D × F	ns	ns	ns	ns	ns	ns

T_s, 50:50 and 66:34 indicating thyme sole culture and intercropping ratio of thyme with soybean; I₂₀, I₅₀ and I₈₀ indicating non-stressed, moderate and severe water deficit levels ns, * and ** indicated no significant difference, significant at 5% probability level and significant at 1% probability level, respectively.

Table 5. The macro- and micro-nutrients concentrations of thyme in different treatments.

Treatments	N content (g kg ⁻¹)		P content (g kg ⁻¹)		K content (g kg ⁻¹)		Ca content (g kg ⁻¹)		Mg content (g kg ⁻¹)		Fe content (mg kg ⁻¹)		Zn content (mg kg ⁻¹)	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
Cropping Pattern														
Sole culture	17.1 b	18.7 c	1.95 a	2.1 a	13 b	14.6 b	4.2 ab	5.1 b	2.4 b	2.9 b	310.1 b	401.5 c	18.04 a	21.2 b
50:50	19.6 a	22.5 a	2.05 a	2.3 a	15.2 a	17.1 a	4.4 a	5.3 a	2.58 ab	3.3 a	377.8 a	503.8 a	18.96 a	23.3 a
66:34	18.6 a	21.1 b	1.96 a	2.2 a	15.1 a	16.7 a	4.1 b	5.2 ab	2.61 a	3.4 a	329.7 b	448.2 b	18.27 a	20.8 b
LSD	1.08	0.91	0.11	0.27	1.12	1.15	0.22	0.18	0.15	0.21	32.63	34.04	1.07	1.21
Fertilization														
Control	17.5 b	19.8 b	1.9 b	2.3 b	13.5 b	15.1 b	3.98 b	5.04 b	2.3 b	2.98 b	311.1 b	420.5 b	17.87 b	20.9 b
AMF	19.4 a	21.7 a	2.1 a	2.5 a	15.4 a	17.1 a	4.43 a	5.33 a	2.8 a	3.40 a	367.3 a	481.8 a	18.99 a	22.6 a
LSD	0.88	0.74	0.09	0.11	0.91	0.94	0.18	0.14	0.16	0.17	26.65	27.80	0.87	0.99
Drought stress														
I ₂₀	22.25 a	24.7 a	2.35 a	2.82 a	18.02 a	19.9 a	4.8 a	5.8 a	3.04 a	3.66 a	408.2 a	532.8 a	20.9 a	25.1 a
I ₅₀	18.1 b	20.2 b	1.96 b	2.3 b	14.1b	15.7 b	4.2 b	5.1 b	2.51 b	3.16 b	318.2 b	436.7 b	18.5 b	21.5 b
I ₈₀	15.04 c	17.4 c	1.65 c	1.99 c	11.29 c	12.8 c	3.7 c	4.7c	2.05 c	2.75 c	291.2 b	384.1 c	15.9 c	18.7 c
LSD	1.08	0.91	0.11	0.14	1.12	1.15	0.22	0.18	0.20	0.21	32.63	34.04	1.07	1.21
Significance														
C	**	**	ns	ns	**	**	*	*	*	**	**	**	ns	**
F	**	**	**	**	**	**	**	**	**	**	**	**	*	**
D	**	**	**	**	**	**	**	**	**	**	**	**	**	**
C × F	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
C × D	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	*
D × F	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
C × D × F	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Ts, 50:50 and 66:34 indicating thyme sole culture and intercropping ratio of thyme with soybean; I₂₀, I₅₀ and I₈₀ indicating non-stressed, moderate and severe water deficit levels

ns, * and ** indicated no significant difference, significant at 5% probability level and significant at 1% probability level, respectively.

Table 6. Proportion of thyme essential oil constituents in different cropping patterns and irrigation levels with AMF application (first year).

No	Components	RI ^a	LIT RI ^b	Treatments ^c												
				T _s	T _s	T _s	T _s	T _s	T _s	50:50	50:50	50:50	50:50	50:50	50:50	66:34
				M ₀	M ₀	M ₀	M ₁	M ₁	M ₁	M ₀	M ₀	M ₀	M ₁	M ₁	M ₁	M ₀
				I ₂₀	I ₅₀	I ₈₀	I ₂₀	I ₅₀	I ₈₀	I ₂₀	I ₅₀	I ₈₀	I ₂₀	I ₅₀	I ₈₀	I ₂₀
1	α -Thujene	921	924	0.54	0.69	0.59	0.62	0.54	0.67	0.62	0.52	0.69	0.53	0.56	0.77	0.51
2	α -Pinene	931	932	0.88	0.73	0.79	0.75	0.53	0.66	0.64	0.32	0.68	0.57	0.47	0.51	0.67
3	Camphene	944	946	6.89	7.21	7.12	8.42	8.29	7.92	6.52	7.11	7.54	8.51	7.73	7.62	7.23
4	Sabinene	968	969	0.67	0.56	0.54	0.18	0.12	0.16	0.74	0.65	0.57	0.24	0.16	0.07	0.78
5	1-Octen-3-ol	972	974	0.24	0.22	0.14	0.26	0.18	0.15	0.09	0.05	0.19	0.13	0.09	0.23	0.22
6	β -Myrcene	985	988	2.26	1.79	2.12	2.31	2.04	2.11	2.15	1.74	1.83	2.04	2.12	1.98	1.97
7	3-Octanol	997	998	tr.	-	0.04	-	-	tr.	-	-	-	-	tr.	-	tr.
8	α -Terpinene	1011	1014	0.69	0.49	0.43	0.67	0.43	0.69	0.72	0.34	0.52	0.75	0.29	0.39	0.66
9	<i>p</i>-Cymene	1019	1020	12.74	11.37	12.96	13.04	12.90	13.61	12.78	12.04	13.81	13.17	12.15	13.87	12.94
10	Limonene	1022	1024	0.51	0.45	0.26	0.49	0.43	0.19	0.39	0.19	0.53	0.50	0.23	0.22	0.37
11	1,8-Cineole	1024	1026	5.83	4.74	5.05	4.62	3.67	3.84	5.12	4.61	4.78	4.27	2.94	3.57	5.22
12	<i>g</i>-Terpinene	1052	1054	13.80	15.12	14.49	13.14	16.07	13.83	14.64	17.22	17.10	15.59	17.03	17.48	13.89
13	<i>cis</i> -Sabinene hydrate	1064	1065	0.53	0.59	0.61	0.41	0.63	0.54	0.47	0.36	0.39	0.40	0.28	0.19	0.37
14	α -Terpinolene	1066	1068	0.08	0.14	0.18	0.15	0.16	0.21	0.11	0.11	0.16	0.11	0.09	0.18	0.14
15	Linalool	1094	1095	3.17	2.43	2.16	2.19	1.71	1.83	2.91	1.88	2.08	1.87	1.71	1.37	2.37
16	Camphor	1137	1141	5.37	5.14	4.62	5.61	5.16	5.60	4.96	4.32	3.74	4.21	4.61	4.12	5.22
17	Borneol	1161	1165	1.19	1.07	0.84	1.35	1.11	1.21	0.96	1.11	0.93	1.20	1.09	0.92	1.05
18	Terpinen-4-ol	1171	1174	0.19	0.21	0.34	0.44	0.58	0.51	0.22	0.56	0.44	0.36	0.50	0.74	0.42
19	Carvacrol, methyl ether	1238	1241	1.27	1.37	1.23	1.12	1.31	1.16	1.19	1.52	1.43	1.13	1.39	1.19	1.40
20	Geranial	1260	1264	0.16	0.09	0.14	0.20	0.08	0.14	0.07	tr.	0.04	0.13	0.09	0.08	0.06
21	Thymol	1287	1289	31.12	32.51	33.10	31.19	32.12	34.19	33.52	36.91	31.89	32.12	37.26	34.79	33.10
22	Carvacrol	1297	1298	2.13	2.78	2.35	2.45	2.63	2.01	2.39	2.73	2.94	2.71	2.83	3.18	2.26
23	Thymol acetate	1347	1349	0.49	0.44	0.51	0.29	0.18	0.26	0.53	0.58	0.47	0.21	0.24	0.25	0.46
24	(E)-Caryophyllene	1415	1417	3.81	3.66	3.54	4.19	3.66	3.98	3.91	2.72	3.65	3.91	2.72	3.71	4.08
25	α -Humulene	1451	1452	tr.	-	-	-	-	tr.	0.06	-	-	-	-	-	-
26	Germacrene D	1481	1484	0.39	0.32	0.26	0.38	0.21	0.11	0.44	0.19	0.25	0.56	0.17	0.26	0.49
27	δ -Cadinene	1521	1522	0.79	0.63	0.66	0.61	0.44	0.37	0.54	0.37	0.42	0.58	0.21	0.29	0.56
28	Caryophyllene oxide	1581	1582	2.04	1.72	1.97	1.86	1.73	1.91	1.76	1.53	1.61	1.67	1.24	1.28	2.12
29	β -Bisabolene	1766	1768	-	-	tr.	-	-	-	-	-	-	-	-	-	-
	Total identified (%)			98.85	98.47	98.04	97.44	97.91	98.92	99.45	99.7	98.68	98.47	98.73	98.86	99.56

Table 1. Chemical composition of the essential oil of <i>Thymus serpyllifolius</i> L.														
Grouped compounds (%)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Monoterpene hydrocarbons	37.98	39.41	39.3	41.62	42.35	38.84	39.2	39.13	43.33	41.3	42.24	42.77	38.02	
Oxygenated monoterpenes	52.53	52.51	52.13	50.52	50.34	53.51	53.45	54.71	49.29	49.72	53.03	50.58	53.07	
Sesquiterpene hydrocarbons	5.03	4.61	4.46	5.18	4.31	4.49	4.95	3.28	4.32	5.05	3.10	4.26	5.13	
Oxygenated sesquiterpenes	2.04	1.72	1.97	1.86	1.73	1.91	1.76	1.53	1.61	1.67	1.24	1.28	2.12	
Others	0.27	0.22	0.18	0.26	0.18	0.18	0.09	0.05	0.19	0.13	0.12	0.23	0.22	

^a RI, linear retention indices on DB-5 MS column, experimentally determined using homologue series of *n*-alkanes; ^b Relative retention indices taken from Adams.

^c Ts, 50:50 and 66:34 indicating thyme sole culture and intercropping ratio of thyme with soybean; I₂₀, I₅₀ and I₈₀ indicating non-stressed, moderate and severe water deficit levels; M₀: non-usage of AMF, M₁: usage of AMF

^d Identification methods: MS, by comparison of the mass spectrum with those of the computer mass libraries Wiley, Adams and NIST 08; RI, by comparison of retention index with those reported in Adams and NIST 08; Std, by comparison of the retention time and mass spectrum of available authentic standard.

Table 7. Proportion of thyme essential oil constituents in different cropping patterns and irrigation levels with AMF application (second year).

No	Components	RI ^a	LIT RI ^b	Treatments ^c												
				T _s	T _s	T _s	T _s	T _s	T _s	50:50	50:50	50:50	50:50	50:50	50:50	66:34
				M ₀	M ₀	M ₀	M ₁	M ₁	M ₁	M ₀	M ₀	M ₀	M ₁	M ₁	M ₁	M ₀
				I ₂₀	I ₅₀	I ₈₀	I ₂₀	I ₅₀	I ₈₀	I ₂₀	I ₅₀	I ₈₀	I ₂₀	I ₅₀	I ₈₀	I ₂₀
1	α -Thujene	921	924	1.02	1.06	0.90	0.80	0.88	0.98	1.05	0.48	1.14	1.26	0.41	1.51	0.98
2	α -Pinene	931	932	1.63	0.98	1.16	1.12	0.91	0.99	0.92	0.77	1.01	0.82	0.67	0.91	1.04
3	Camphene	944	946	1.47	1.73	1.52	1.79	1.43	1.82	1.48	1.14	1.07	1.45	1.28	1.21	1.38
4	Sabinene	968	969	0.46	0.29	0.51	0.69	0.39	0.51	0.61	0.37	0.32	0.72	0.31	0.26	0.83
5	1-Octen-3-ol	972	974	0.71	0.55	0.58	0.32	0.21	0.22	0.42	0.38	0.52	0.30	0.17	0.14	0.62
6	β -Myrcene	985	988	1.69	1.75	1.68	1.56	1.43	1.62	1.84	1.37	1.59	1.46	1.26	1.57	1.55
7	3-Octanol	997	998	tr.	0.05	-	tr.	-	0.05	-	-	-	-	-	tr.	tr.
8	α -Terpinene	1011	1014	0.93	0.68	0.83	0.81	0.51	0.67	0.79	0.42	0.73	0.45	0.18	0.43	0.64
9	<i>p</i>-Cymene	1019	1020	14.07	13.73	14.04	14.48	13.76	14.79	14.86	14.12	15.44	14.09	13.85	16.06	14.89
10	Limonene	1022	1024	0.67	0.42	0.54	0.61	0.51	0.79	0.50	0.23	0.61	0.59	0.19	0.72	0.88
11	1,8-Cineole	1024	1026	1.16	1.41	1.37	0.97	1.12	1.17	1.19	0.99	1.15	0.83	0.94	0.96	1.27
12	<i>g</i>-Terpinene	1052	1054	16.93	17.96	18.12	18.42	18.06	19.12	17.02	18.46	19.14	18.54	20.88	21.55	17.13
13	<i>cis</i> -Sabinene hydrate	1064	1065	0.97	0.94	0.92	1.39	0.86	0.86	1.18	0.69	0.83	0.91	0.68	0.77	0.88
14	α -Terpinolene	1066	1068	0.19	0.12	0.15	0.12	0.08	0.07	0.41	0.09	0.11	0.09	0.09	0.03	0.14
15	Linalool	1094	1095	1.82	1.59	1.82	2.16	1.86	2.02	1.81	1.68	1.64	1.95	1.88	1.85	1.87
16	Camphor	1137	1141	0.42	0.20	0.26	0.22	0.11	0.22	0.19	0.19	0.20	0.19	0.09	0.07	0.18
17	Borneol	1161	1165	1.94	2.12	1.67	1.85	1.37	1.04	2.11	1.67	1.64	1.49	1.15	1.23	2.09
18	Terpinen-4-ol	1171	1174	0.53	0.39	0.26	0.52	0.19	0.16	0.93	0.34	0.31	0.46	0.07	0.21	0.73
19	Carvacrol, methyl ether	1238	1241	0.82	0.92	0.77	0.59	0.38	0.43	0.78	0.96	0.89	0.48	0.53	0.31	0.78
20	Geranial	1260	1264	0.29	0.18	0.13	0.12	0.07	0.06	0.09	tr.	0.08	0.09	-	tr.	0.09
21	Thymol	1287	1289	40.89	42.23	42.46	41.17	46.29	44.09	41.32	48.15	44.12	42.71	48.59	43.13	42.28
22	Carvacrol	1297	1298	2.95	3.62	3.51	3.03	3.16	3.88	3.42	3.58	3.68	3.33	3.52	3.77	3.01
23	Thymol acetate	1347	1349	0.08	0.06	0.06	0.11	0.12	0.14	0.12	0.11	0.38	0.12	0.20	0.13	0.02
24	(E)-Caryophyllene	1415	1417	1.72	1.27	0.93	2.19	1.65	0.92	1.49	0.59	0.99	2.13	1.82	1.37	1.58
25	α -Humulene	1451	1452	0.09	0.05	0.04	-	-	-	0.05	tr.	-	0.11	tr.	-	-
26	Germacrene D	1481	1484	0.72	0.86	0.41	0.27	0.12	0.45	0.87	0.29	0.23	0.59	0.06	0.23	0.94
27	δ -Cadinene	1521	1522	1.19	0.63	0.92	0.33	0.16	0.14	0.42	0.26	0.18	0.34	0.12	0.18	0.42
28	Caryophyllene oxide	1581	1582	1.68	0.94	1.48	0.69	0.62	0.76	1.26	0.76	1.06	0.71	0.59	0.36	1.04
29	β -Bisabolene	1766	1768	0.08	0.04	tr.	-	-	0.05	0.05	-	tr.	-	-	-	tr.
	Total identified (%)			98.14	98.08	98.06	97.35	99.27	99.02	98.18	98.16	99.09	97.21	98.56	99.29	98.32

	Grouped compounds (%)													
	Monoterpene hydrocarbons	37.87	38.91	38.3	40.28	40.88	41.29	38.07	37.36	41.05	39.38	40.03	44.06	38.32
	Oxygenated monoterpenes	53.06	54.78	54.38	53.25	53.61	55.14	54.55	58.49	55.03	53.65	56.74	52.76	54.34
	Sesquiterpene hydrocarbons	3.80	2.85	2.32	2.54	1.95	1.56	2.88	1.17	1.43	3.17	2.03	1.78	2.96
	Oxygenated sesquiterpenes	1.68	0.94	1.48	0.69	0.62	0.76	1.26	0.76	1.06	0.71	0.59	0.36	1.04
	Others	0.73	0.60	0.58	0.34	0.21	0.27	0.42	0.38	0.52	0.3	0.17	0.17	0.66

^a RI, linear retention indices on DB-5 MS column, experimentally determined using homologue series of *n*-alkanes; ^b Relative retention indices taken from Adams.

^c Ts, 50:50 and 66:34 indicating thyme sole culture and intercropping ratio of thyme with soybean; I₂₀, I₅₀ and I₈₀ indicating non-stressed, moderate and severe water deficit levels; M₀: non-usage of AMF, M₁: usage of

^d Identification methods: MS, by comparison of the mass spectrum with those of the computer mass libraries Wiley, Adams and NIST 08; RI, by comparison of retention index with those reported in Adams and NIST 08; Std, by comparison of the retention time and mass spectrum of available authentic standard.

Figures

Figure 1

Sketch of soybean/thyme planting patterns.

Figure 2

The root AMF colonization of in different water deficit levels and planting patterns.

Figure 3

The dry yield of thyme in different water deficit levels and planting patterns.

Figure 4

The Zn content of thyme leaves in different water deficit levels and planting patterns.

Figure 5

The essential oil percentage of thyme in different planting patterns and application of AMF.

Figure 6

The essential oil yield of thyme in different water deficit levels and planting patterns.