Variation of Chemical composition, antioxidant and total phenols of essential from thyme (Origanum syriacum L.) grown under open field conditions and protected soilless condition

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ABSTRACT

The influences of cultivation methods (protected soilless system vs. open field) on the quality and quantity of essential oil yield and composition, antioxidant activity by free radical scavenging activity (antiradical), total phenols as mg Gallic acid equivalents (GAE)/g and flavonoids as mg Catechin Equivalent (mg CE/g of dry weight) in the aerial parts of a Thyme (Origanum syriacum L.) were evaluated in this study. Results obtained showed that the highest essential oil yield (w/w) was obtained under protected soilless conditions (3.52%) which significantly high than that obtained under open field condition (2.11%). Four major compounds were identified in this study including thymol, α- terpinene, p-cymene and linalool. The major compound found was thymol had a concentration of (72.4 μg/g) under protected soilless conditions, which is significantly high than that under open field conditions (50.0 μg/g). α- terpinene was the other major compounds of volatile oils of thyme which varied from (12.7 μg/g) under protected soilless conditions, while open field conditions (7.14 μg/g). The total phenolic content and the antioxidant activity of plant extract were determined, by Folin-Ciocalteau and the 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays respectively. Phenolic content of plant extract of leaves part O. syriacum was significantly (101.9 mg GAE/g DW) under protected soilless conditions which is significantly high than that under open field conditions (81mg GAE/g DW). Antiradical of leaves and shoot parts under protected soilless condition were highly significant than antiradical under open field conditions The presence of total flavonoids in leaves parts of thyme plant was significantly (96 mg GAE/g DW) under protected soilless conditions which is significant than that under open field conditions (58mg GAE/g DW).

Key words: Thyme, Essential Oil, Phenolic, Antioxidant Activity, Flavonoids, protected Soilless

INTRODUCTION

Medicinal and aromatic plants are presently widely studied and used for their large therapeutic potential and benefits as natural preservatives. In this regard, it is well known these medicinal plants contain a complex mixture of bioactive compounds covering a number of demands for human health. A great number of aromatic and medicinal plants contain chemical compounds exhibiting different biological properties and activities [12].

Thyme (Origanum syriacum L.) is one of the most important commercial crops which have a medicinal and aromatic properties, it is a perennial shrub belongs to family Lamiaceae [3], found as wild plant and it is native to Mediterranean regions (e.g. Jordan). Moreover, O. syriacum recently has a great interest as potential therapeutic substances and as natural additives to replace synthetic products in food industry [6,11,17].

Nowadays, O. syriacum was cultivated as an importation medicinal plant due to the different uses such as pharmaceutical, sanitary, cosmetic, and agricultural and food industries over the entire world. The demand for essential oils from thyme is increasing and have attracted a great deal of scientific interest due to their potential as a source of natural antioxidants and biologically active...
compounds [9,25,32] also for perfumery, cosmetic and medicinal applications [14]. Moreover, natural antioxidants and biologically active compounds of thyme plants are used as food additives, pigments, dyes, insecticides, perfume [1]. The importance of aromatic plants such as thyme as natural antioxidants has been determined [8].

Several factors influence the chemical composition of plant essential oils, including genetic structure, climatic factors and the cultural practices such cultivation methods (example; soilless culture [4,5] plant organs like leaf, stem and their developmental stages like pre flowering and after flowering [20] the harvesting season [22,7] developmental stage of collected plant materials [13] and environmental factors, such as light and moisture content, have strong effects on essential oil production [4,5]. Variation in chemical composition of essential oils, in particular, and extracts of thyme was observed in this study. There are many previous studies conducted on the biological activities of essential oils for various medicinal and aromatic plants under conventional method of cultivation. However, studies on the biological activities of medicinal plants are scarce in the literature under protected soilless conditions. Moreover, Al-Tawha [4] and Al-Tawaha [5] were reported the comparison between traditional cultivation and soilless effects of the antioxidant, total phenols, and flavonoids in terms of some quality characteristics and comparison between cultivation methods for sage and spearmint plants.

Essential oil accumulation and compositions of aromatic plants has been reported to be affected by cultivation method like protected soilless condition vs. open filed condition. It was important to know the effect of cultivation method like protected soilless conditions and open field conditions on total phenol, total flavonoid and antioxidant properties for medicinal plants like thyme plants. Soilless culture considered one of the main components of sustainable protected horticulture. It is desirable to develop techniques of agronomical cultivation to improve essential oil products and their specific compounds. Essential oil accumulation and compositions of aromatic plants has been reported to be affected by cultivation method like soilless vs. soil-based cultivation. To our knowledge, few investigations have been made to the chemical constituents of essential oils for thyme leaves under protected soilless condition, although this cultivation method has more yield than soil based conditions [2]. Therefore, the first aim of this study, were to evaluate the effects of cultivation method as well as environmental conditions on chemical compounds for thyme. In addition, few reports were investigated about nutritional, antioxidant, total phenols, flavonoids of thyme cultivated under soilless culture conditions. So, the second aims of this study was to evaluate the total flavonoid content, total phenols of thyme, antioxidant activity under open field and protected soilless conditions as well as methanol extracts, and air drying methods.

Material and Methods

This study was carried out in Jordan University of Science and Technology (JUST) campus during the growing season 2010-2011 in the open field and protected soilless conditions.

2.1. Preparation method for analysis:

2.1.1. Drying methods of aerial parts:

Drying is the most important step in the preparation of thyme leaves for extraction and isolation essential oil by different methods. The samples of thyme plants under soilless cultivation condition were collected from four harvesting times and the samples from soil-based conditions were collected from two harvesting times. The samples were dried at room temperature for 15days. As many other food process, drying needs to be optimized with respect to process economics and product quality which has led to the development of new drying techniques. Drying may introduce undesirable changes in appearance, texture, flavor and colour that are not in agreement with the increasing demand of consumers for the highest quality finished product.

2.2. Plant material:

This study was carried out in Jordan University of Science and Technology (JUST) campus during the growing season 2010-2011 in the open field and soilless culture conditions. Seedling of thyme (Origanum syriacum L.) was cultivated under open field and soilless culture conditions. Seedling of thyme were transplanted in concrete blocks (95*100*75) in cm (W*L*D) filled with soil mix with peatmoss under soil conditions. Peat moss was applied at 33L/m² at the top layer of soil (20cm) to improve for water holding capacity of soil. Inorganic NPK fertilizer has formula N₂, P₂O₅ and K₂O with (20:20:20) ratio was applied at the rate 30 g/m² at the planting time. After each harvest of thyme plants, 25g/m² of NPK fertilizer were added. In addition, 6 g/m² nitrogen fertilizer was added in the form of urea (46%N) after each harvest. Under soilless culture conditions, seedling of thyme was transplanted in woods beds (120*110*25 D) in cm (W*L*D), filled with tuff zeolite (Ø 3-8mm). The irrigation water and nutrients were delivered to the plants via drip irrigation twice a day for 15 minute (early morning and evening).

2.3. Isolation of essential oil:

2.3.1. Preparation of the methanolic extracts:

The extraction procedure for phenolic compounds was based on perva-Uzunlic et al with modifications, where about 0.5grams (three replicates) of each plant sample was weighed out,
and extracted with 50ml of methanol. Extraction was carried out under shaker at overnight at 30°C. Each extract was filtered into a 50 ml volumetric flask using Whatman filter paper No 42. Volume were completed to mark, and allowed to set in the dark until analysis.

2.4. Determination of total phenolic content:

The total phenolic content of the solvent extracts was determined by the method using Folin–Ciocalteu reagent and Gallic acid as standard to produce the calibration curve [23]. 2000 μl of the plant extract (triplicate) were transferred into a test tube, and then mixed with 2.5ml of 10% Folin-Ciocalteu reagent. After 3 minutes for allowing the reaction to take place, 2000 μl of a 10 sodium carbonate (Na₂CO₃) was added. The tube were allowed to stand for 1 hour at ambient temperature, and the absorption was measured at 760 nm using UV-VIS spectrophotometer (model SpectroScan 50) against a blank, with contained 50μl of methanol in place of sample. Gallic acid was used as calibration standard, and results were calculated as Gallic acid equivalent (GAE)/(mg/100g dry weight basis).

2.5. Determination of total flavonoids:

The total flavonoid content of the extract was determined by the method described in the Chinese Pharmacopoeia (Zhishen, 1999). The total flavonoid content of thyme plant under soilless culture and soil conditions was determined. Each sample (0.5ml) of the plant extract (triplicate) were transferred into with 150μl of a NaNO₂ solution (15%). After 6 minutes for allowing the reaction take place, 150 μl of an Aluminum chloride (AlCl₃) solution (10%) was added and allowed to stand for 6 minutes, then 200μl of Sodium hydroxide (NaOH) solution (4%) was added to mixture 0.2ml of distilled water was added to bring volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 minutes. The tubes were allowed to stand for 1 hour at ambient temperature, and absorption was measured at 510nm using spectrophotometer against a blank which contain 50 ml of methanol in place sample. Catechin was used as calibration standard with different concentrations 10, 30, 80, 100, 150, 200, 300, 400 and 500mg/l were tested to obtain standard curve; results were expressed as Catechin equivalents (mg Catechin/g dried extract).

2.6. Evaluation of antioxidant (antiradical) activity:

Antioxidant activity was determined according to Brand-Williams, 199. Briefly, Free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging effect was determined. The DPPH were soluble in methanol. Fresh DPPH stock solution (25ml) was prepared daily. The solution was prepared by weighing 50mg/100 ml which represent the amounts we need, then 0.0125g of DPPH was dissolved in 25 ml methanol which resulted in purple color solution. The mixture was mixed thoroughly and allowed to stand in the dark for 60 minutes. Absorbance at zero time (Ato) at 517nm wavelength was determined. Absorbance then was read at 517 nm, against the blank. The percentage inhibition of DPPH free radical was calculated by the formula:

\[
\text{Percentage inhibition} \% = \left( \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100
\]

Where, A blank is the absorbance of control reaction (DPPH alone) and A sample is the absorbance of DPPH solution in the presence of the test compound. IC 50 values denote the concentration of the sample, required to scavenge % of DPPH free radicals. Extract concentration providing 50% inhibition (IC50) was calculated from the plot of inhibition percentage against extract concentration. All determinations were carried out in triplicate and the results were averaged.

2.7. Statistical analysis:

All Data were statistically analyzed using analysis of variance (ANOVA) according to the statistical package MSTAT-C (Michigan State Univ., East Lansing, MI, USA). Probabilities of significance among treatments and LSD (P≤ 0.05) were used to compare means among treatments.

3. Results:

3.1. Effect of protected soilless condition and open field condition on yield essential oil:

The essential oil yield of thyme (Origanum syriacum L.) was influenced by cultivation methods (protected soilless condition vs. open field conditions). The variation of the essential oil Origanum syriacum aerial part with effect two model of cultivation thyme are shown in Table 1. The highest essential oils percentage (relative to the amount of dried herbs used) was noticed under protected soilless (3.52%) which was significantly higher than the essential oils percentage that was achieved under open field conditions (2.11%).

3.2. Effect of protected soilless condition and open field condition chemical composition of essential oils:

Results which that obtained by the GC-MS chemical analysis of thyme (Origanum syriacum L.) are presented in Table 1. The result shows that thymol, \(\gamma\)-terpinene and p-cymene are the major components under protected soilless conditions and open field conditions (Table 1).
It can be noticed from Table 1 that thymol, γ-terpinene and p-cymene contents varied significantly, while linalool was not significant. The major constituent was thymol followed by γ-terpinene, p-cymene and linalool. The results revealed clear variation in the chemical composition of essential oils for thyme which was grown under open field and protected soilless conditions. The results showed that thymol concentration was (72µg /g) under protected soilless condition which is significantly higher than that under open field cultivation condition (50.0 µg/g). Few studies about constitutes for essential oils for thyme plant under soilless condition. Almost of results was conducted under open filed condition.

γ-terpinene and p-cymene concentration under protected soilless condition were 12.7 µg/g and 7.6 µg/g which were significantly higher than that concentration obtained under open field (7.14 µg/g) and (3.70 µg/g). Linalool concentration was not significant and the values varied from (1.33 µg/g) under soilless cultivation to (1.77 µg/g) under soil cultivation, but in all cases thymol, γ-terpinene and p-cymene were the most abundant compounds found to be major compounds in our study and γ-terpinene and p-cymene classified as the biosynthetic precursors of monoterpenoid phenols. While results of Rasooli and Mirmostafa [18], showed thymol being the third major component in the wild thyme oil, after the content of γ-terpinene and p-cymene. Many studies were conducted on the chemical composition of the oils from the plants belonging to the genus Thymus, including *T. serpyllum*, *T. algeriensis* and *T. vulgaris* [15,19]. Sfaii-Ghomi et al. [21], where γ-pinene and carvacrol were reported to be the major oil components. Besides the thymol (30%), carvacrol (20%) was reported to be the second main component of the wild thyme oils [26], while results of Rasooli and Mirmostafa [18], showed thymol being the third major component in the wild thyme oil, after th content of γ-terpinene and p-cymene.

The comparison reports of the present results of essential oils composition of thyme with other results was reported under soil conditions. While, to comparison the present results of essential oils composition under protected soilless conditions there were few reports available. Al-Tawaha et al [4,5] reported that the essential oils and chemical composition were variation under soilless condition for chemical composition of *S. officinalis* and *M. spicata* essential oils which were varied significantly with cultivation conditions. Al-Tawaha et al [4] reported that cultivation *S. officinalis* under protected soilless conditions was important for major compounds. In other study, Al-Tawaha et a [5], reported that *M. spicata* grown under protected soilless condition was rich in essential oil and some constituents such pulegone and 1,8 cineole content which was proved to be superior in both oil content and quality, in terms of substantial contents of some constituents.

The qualitative and quantitative variations between the present results and those from other parts of the world may be attributed to genetic variability and/or to environmental conditions. Differences in analytical methods can also be responsible. These results clearly indicate that cultivation method should carefully selected to ensure maximum yield of essential and chemical composition.

3.3 Effect of protected soilless condition and open field condition on total phenols, total flavonoids and antioxidant activity:

In this work, both stem and leaf phytochemicals were evaluated under two cultivation method. Different phytochemicals have various protective and therapeutic effects on plants which are essential to prevent diseases. Thyme was analyzed for their phyto constituents under two planting methods (protected soilless vs. open field conditions). Generally, quantitative estimation of the phytochemical constituents of thyme shows that, the medicinal herbs such as thyme is rich in total phenols, total flavonoids and antioxidant activity according to data shown in Table 2,3,4 respectively. Phenols concentration of methanol extracts of different parts (leaves and shoot) for thyme plants were evaluated using the Folin-Ciocalteu reagent and expressed as mg Gallic acid equivalent (GAE/g dry weight) under two planting system. There was great variation between total phenolic concentration of leaves and shoots in thyme plants. As shown in Table (2) the high significant yield of leaves was 101.9 mg GAE/g under soilless cultivation was higher than total phenols of leaves 81 mg GAE/g under soil cultivation. The yield of total phenols of shoot plant was varied between 65 mg GAE/g under soilless cultivation 62 mg GAE/g cultivation soil cultivation. Al-Tawaha et al 2013a and 2013 b reported that the cultivation method (protected soilless vs. open filed) was highly affected on total phenols for sage (*Salvia officinalis* L.) and spearmint (*Mentha spicata* L.) plants.

**Table 1: Essential oils percentage and volatile components (µg/g) identified in thyme plants under soil-based and soilless cultivation.**

<table>
<thead>
<tr>
<th>Cultivation methods</th>
<th>Essential oil% (w/w)</th>
<th>Thymol (µg/g)</th>
<th>γ-Terpinene (µg/g)</th>
<th>p-Cymene (µg/g)</th>
<th>Linalool (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>2.11b</td>
<td>72.4 a*</td>
<td>12.7 a*</td>
<td>3.76</td>
<td>1.77 a</td>
</tr>
<tr>
<td>Soiless</td>
<td>3.52a</td>
<td>2.11b</td>
<td>12.7 a*</td>
<td>3.76</td>
<td>1.77 a</td>
</tr>
<tr>
<td>Significance</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>NS</td>
</tr>
</tbody>
</table>

*: significant at P≤0.05, **: significant at P≤ 0.01.
Table 2: Total phenols and flavonoids of two parts (leaves and shoot) for thyme plants under open filed and soilless culture conditions.

<table>
<thead>
<tr>
<th>Cultivation methods</th>
<th>Total phenols (mg GAE/g)</th>
<th>Total phenols (mg GAE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Shoot¶</td>
</tr>
<tr>
<td>Open field</td>
<td>81 b</td>
<td>62 a</td>
</tr>
<tr>
<td>Soilless culture</td>
<td>101.9 a</td>
<td>65 a</td>
</tr>
<tr>
<td>Significance</td>
<td>*</td>
<td>ns</td>
</tr>
</tbody>
</table>

*: Significant at P≤0.05, **: Significant at P≤ 0.01.
¶ Shoot: leaves + stem

Total concentration of methanol extracts of leaves and shoot for thyme plants were evaluated using the AlCl₃ reagent and expressed as mg catechin equivalent (mg CE/g of dry weight) under two planting system (soil vs. soilless). The presence of total flavonoids in leaf part was significantly high 97 mg CE/g under soilless cultivation than the total flavonoids under soil cultivation 50 mg CE/g.

Table 3: Total flavonoids of two parts (leaves and shoot) for thyme plants under open filed and soilless culture conditions.

<table>
<thead>
<tr>
<th>Cultivation methods</th>
<th>Flavonoids (mg CE/g)</th>
<th>Flavonoids (mg CE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Shoot¶</td>
</tr>
<tr>
<td>Open field</td>
<td>58 b</td>
<td>48 a</td>
</tr>
<tr>
<td>Soilless culture</td>
<td>96 a</td>
<td>39 a</td>
</tr>
<tr>
<td>Significance</td>
<td>*</td>
<td>ns</td>
</tr>
</tbody>
</table>

*: Significant at P≤0.05, **: Significant at P≤ 0.01.
¶ Shoot: leaves + stem

The total flavonoids of shoot parts were varied between 39 mg CE/g under soilless cultivation and 48 mg CE/g under soil cultivation and no significance between them. Al-Tawaha et al. 2013a and 2013 b reported that the cultivation method (protected soilless vs. open filed) was highly affected on total flavonoids for sage (Salvia officinalis L.) and spearmint (Mentha spicata L.) plants.

The antioxidant activity of methanol plant extract was evaluated according to their ability for scavenging free radicals by using DPPH. The antiradical (1/antioxidant) concentration varied highly significant in leaves and high significant in shoot of soilless cultivation than in soil cultivation. Antiradical of leaf under soilless cultivation was 5 which are highly significance than antiradical of leaves under soil cultivation 4. In other part, the antiradical (1/antioxidant) in shoot was high under soilless and values of antiradical was 5 and the values under soil cultivation was 3. A strong relationship between total phenols contents and antioxidant activity has been reported by several studies reported that phenolic compounds in spices and herbs significantly contributed to their antioxidant properties. Results in this study are in agreement with those reported by Shan et al. in that the most common flavonoids are mainly distributed in the sage, thyme and Labiatae family in general. Antioxidant activity of leave part for thyme plants was high significance under soilless than under soil cultivation. Similarly, antioxidant for shoot part under soilless cultivation conditions had highly significantly than soil cultivation.

Table 4: Antioxidant activity in two parts (leaves and shoot) for thyme plants under open filed and soilless culture conditions

<table>
<thead>
<tr>
<th>Cultivation methods</th>
<th>Antiradical (1/antioxidant) mg/g</th>
<th>Antiradical (1/antioxidant) mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Shoot¶</td>
</tr>
<tr>
<td>Open field</td>
<td>4.3 b</td>
<td>3.1 b</td>
</tr>
<tr>
<td>Soilless culture</td>
<td>5.2 a</td>
<td>4.6 a</td>
</tr>
<tr>
<td>Significance</td>
<td>**</td>
<td>*</td>
</tr>
</tbody>
</table>

*: Significant at P≤0.05, **: Significant at P≤ 0.01.
¶ Shoot: leaves + stem.

Total flavonoid concentration of leaf part for thyme plants was significantly high under soilless cultivation than that of soil cultivation, but in shoot it was not significance. In comparisons of antioxidant activities of leaf part for thyme plants under two cultivation methods it was showed highly significant value under soilless cultivation than that soil cultivation. A strong relationship between total phenols contents and antioxidant activity has been reported by several studies reported that phenolic compounds in spices and herbs significantly contributed to their antioxidant properties. Result clearly showed that had the highest total phenolic content

Conclusions:
The present study is the first report on the composition of the essential oil from leaves and stems of thyme (Origanum syriacum L.) growing under protected soilless culture. In conclusion,
cultural practices factors; in combination with environmental conditions appear to be affected on antioxidant activities, total phenolic content, total flavonoids levels in leaves and shoot of thyme (*Origanum syriacum* L.) plants. Comparison of protected soilless vs. open filed giving understanding how cultural practices combination with environmental factors affect the variation in antioxidant content of *O. syriacum* plants could represent a significant factor toward optimizing growth conditions for maximal recovery of phytochemicals and antioxidants. In conclusion, this study provides new knowledge for effect cultivation methods with combination environmental conditions on chemical compositions essential oils for thyme (*Origanum syriacum* L.) plant. To produce the best yield of essential oils with a good concentration, it is necessary to combine suitable cultivation methods that optimized the concentration of volatile compounds. Essential oil composition for open field and ported soilless conditions is very variable, it is important that cultivation methods be optimized for some particular volatile compound than others. The major compound found was thymol under protected soilless conditions, which is significantly high than that under open field conditions. Selection for cultivation methods should be done according to the target compound and therapeutic value of sage. The presence of biologically active molecules as major components in leaf oil makes it of high importance for medical purposes. Hence, more investigations are needed to study its characteristics and its possible uses.

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References


