



Full Length Article

Essential Oil Content and Heavy Metals Composition of *Thymus vulgaris* Cultivated in Various Climatic Regions of Jordan

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ABSTRACT

This study was conducted to estimate the concentration of essential oil and some heavy metals (Cd, Pb, Ni, Mn, Zn & Cu) in thyme plant (*Thymus vulgaris*) cultivated in the southern (Maan, Al-Shouback & Al-Tafeileh), middle (Amman) and northern (Jeresh, Ajlune & Irbid) regions of Jordan. The results showed a wide variation of essential oil values yielded from thyme plant. The mean value of essential oil was 4.63% at the north environment, while it was decreased to 2.77 and 2.70% in the south and middle regions, respectively. The results showed differences in the metal concentrations of all samples. The highest concentrations (in mg kg⁻¹) of Ni (22.21), Cu (7.97) and Zn (48.73) were recorded at the north environment of Jordan but they were within permissible limit for medicinal plants. Cadmium (Cd) in all specimens of *T. vulgaris* collected from the completely cultivated areas of Jordan was non-detectable (<1.20 mg kg⁻¹). The average concentrations of Pb were 33.31, 22.38, 29.46 mg kg⁻¹, at the north, middle and south cultivated areas, respectively which were higher than permissible limits. In conclusion the essential oil and heavy metals contents in *T. vulgaris* are mainly affected by variable natural climatic conditions.

Key Words: Amman; Cadmium; Environment; Medicinal plant; Lead; Thyme

INTRODUCTION

Thyme (*Thymus vulgaris* L.) is a herbaceous perennial plant belonging to the *Lamiaceae* family. The Plant is native to the western Mediterranean region and southern Italy (Matiljan, 2008). There are 350 species of thyme cultivated all over the world (Stahl-Biskup & Sáez, 2002); nine of those are distributed in southern and northern areas of Jordan (Khairallah, 2006). Jordan is located 80 km east of the eastern coast of the Mediterranean Sea. Its location between 29° 11'N and 33° 22'N and between 34° 19' E and 39° 18'E with an area of 89329 km², more than 80% is classified as arid areas. The climate in Jordan is predominantly of the Mediterranean type. It comprises hot and dry summer and cool wet winter with two short transitional periods in autumn and spring. Rainfall decreases from North to South, West to East and from higher elevation to lower ones (JordanweatherTM, 2008).

The green part of thyme plant constitutes the most popular herbal medicine and spice, used in Jordan as well as in all other developing countries. Thymol and carvacrol, which are the principal constituents of thyme oil (Atti-Santos *et al.*, 2004) have been reported to act as antioxidant (Kulisic *et al.*, 2005), antimicrobial agent (Deans & Ritchie, 1987), antifungal agent (Klarić *et al.*, 2007) treatment for respiratory tract diseases (Inouye *et al.*, 2001), wound

healing, a stomachic carminative, diuretic, urinary disinfectant and vermifuge (Boskabady *et al.*, 2006). The composition and quantity of essential oil from a particular species of thyme plant could be markedly affected by harvesting season (Atti-Santos *et al.*, 2004), geographical environment and other agronomical factors (Hassanali *et al.*, 2004; Jordan *et al.*, 2006).

The beneficial effects of thyme are well known from ancient times and consumption of its extract is recommended all over the world (Akerle, 1993). It is considered the main ingredient of many phytopreparations and commonly used as water extracts for its pharmacological activities and thus, have a very important role in phytotherapy (Razic *et al.*, 2003). Recently, thyme has become one of the most important medicinal plants used as a natural additive in poultry and livestock feeding studies (Hernandez *et al.*, 2004; Bolukbasi & Erhan, 2007). Such studies showed that thyme plant could be considered as an alternative natural growth promoter for poultry instead of antibiotics (McDevitt *et al.*, 2007).

Trace mineral elements are constitutive plant compounds with biological activity as essential or toxic agents in metabolism. Toxic effects of heavy metals on plants are very complex and can affect pharmacologically active compounds in medicinal plants and thus seriously deteriorate the quality safety and efficacy of natural plant

products (Djukic-Cosic *et al.*, 2007). Therefore, among the other quality control analyses of the raw material of medicinal plants, determination of metals, especially toxic ones, is of special concern since the industrial pollution of agricultural land and forests is becoming a serious ecological issue in many parts of the world (Jones & Case, 1990; Maksimović *et al.*, 1999; Al-Jundi, 2000; Ajasa *et al.*, 2004; Erdemoglu & Basgel, 2006; Al-Alawi & Mandiwana, 2007). The aim of this work was to determine the yields of essential oil and the contents of some toxic heavy metals like Cd and Pb in the herb thyme collected during 2007 from various localities of southern, middle and northern regions of Jordan.

MATERIALS AND METHODS

Plant material. Aerial parts of thyme (*Thymus vulgaris* L.) cultivated under rain fed conditions were collected during May-June 2007 from seven different locations in Jordan; Al-Tafeileh, AL-Shoubak and Maan in the south. Amman in the middle and Jeresh, Ajlune and Irbid in the north. The plant material was dried in draughty place at about 20°C, all specimens were identified on the basis of macroscopic characteristics by comparison with authentic sample and a voucher specimen was deposited at the Herbarium of Al-Shoubak University College. The dried samples of thyme were separately crushed and mild into small pieces and sieved through (0.5 mm) mesh sieve.

Determination of essential oil. Essential oil contents were extracted from dried aerial parts of the all collected samples of thyme plant by the hydro-distillation method using a Clevenger-type apparatus (British Pharmacopeia, 1998) similar to European Pharmacopoeia (EP, 2005), using 50 g of the dried mild and sieved plant and 500 mL of water in 1000 mL round bottomed flask. Distillation time was 2 h at a rate of 2-3 min⁻¹ the values reported are the mean of at least three distillations and three replications for each specimen.

Determination of heavy metals content in thyme plant samples. Heavy metals content of particular thyme samples were analyzed using Atomic Absorption. Flame Emission Spectrophotometer Model AA-6200 (Shimadzu, Japan) as described by (Al-Alawi & Mandiwana, 2007). The plant samples were oven dried at 70°C for 24 h until the dry weight was constant. The dried samples were then ground and passed through a 0.2 mm plastic sieve. Then, 0.5 g of plant sample was wet digested with an Ultra-pure nitric acid (HNO₃ (10-15 mL) in a polyethylene test tube using a heating block digestion unit at 120°C. The final solution was filtered into a 25 or 50 mL volumetric flask through a 45-µm filter paper and diluted to the mark with ultra-pure water. Ultra-pure water was used for all dilutions and sample preparation. Analytical results have evaluated by statistical analysis system (SAS, 1987). The standard error values of the means were calculated to compare the site categories.

RESULTS AND DISCUSSION

The means of essential oil contents in *T. vulgaris* cultivated in various geographical locations in Jordan are presented in Table I. There were wide variations of essential oil values yielded from thyme plant cultivated at different geographical regions in Jordan. The mean value of essential oil was 4.63% at the north environment, while it was decreased to 2.77 and 2.70% in the south and middle cultivated regions, respectively. *T. vulgaris* cultivated at the northern environment recorded higher concentrations than their corresponding values in the middle and south regions. The results clearly showed the effect of geographical cultivated region of thyme plant on the yields of essential oils. Such variations in the yields of essential oils may be related to the differences of cultivated region, geographical and climatic differences (Table. II). Hassanali *et al.* (2004) and Jordan *et al.* (2006) found that wild-growing thyme in Jordan had higher concentrations of essential oil (5.40%) than recorded in Egypt (1.07%), Chili (0.39%), Iran (1.4%) and Belarusian was 1.75% (Karawya & Hifrawy, 1974; Hudaib & Aburjai, 2007; Khazaie *et al.*, 2008). On the other hand, the results showed that all our studied specimens were found to satisfy the requirements of Pharmacopeias such as European Pharmacopoeia (EP), which requires a yield of oil should be ≥ 1.2% v/w. calculated with reference to the anhydrous (dried) drug (EP, 2005).

The results of heavy metals concentrations (mg kg⁻¹) in *T. vulgaris* cultivated at variable natural conditions in different regions of Jordan are shown in Table III. Cadmium (Cd) in all specimens from the completely cultivated areas of Jordan were non-detectable (<1.20 mg kg⁻¹). Till now, no physiological functions of Cd in the human body have been reported. However, Cd intoxication can lead to kidney, bone and pulmonary damages (Godt *et al.*, 2006). The concentrations of Cd in different regions were generally lower than those recommended by World Health Organization (WHO, 1985) in the medicinal plants. It has been already reported that intake of cadmium-contaminated food causes acute gastrointestinal effects, such as vomiting and diarrhea (Nordberg, 2004), and there is some proof that cadmium can cause cancer (Waalkes *et al.*, 1988).

The average concentrations of Pb in thyme from north, middle and south regions of Jordan were 33.31, 22.38 and 29.46 mg kg⁻¹, respectively. The highest mean concentration of Pb (37.82 mg kg⁻¹) was observed in Irbid in the north, while the lowest (22.38 mg kg⁻¹) was recorded in Amman; in the middle regions. Pb profiles recorded in the north and south cultivated areas were Jeresh < Ajlune < Irbid and Maan < Al-Shoubak and Al-Tafeileh, respectively. It was much higher than the maximum level (10 mg kg⁻¹) recommended by WHO in medicinal plants. These results were in agreement with the results obtained by Amr and Đordević, (2000) who found that high contents of Pb (17 to 69 mg kg⁻¹) were detected in the medicinal plant *Salvia officinalis* grown in various regions of Jordan. This high

Table I. Means of essential oils (%) and moisture content (%) of *T. vulgaris* cultivated in different environmental regions in Jordan

	Various cultivated locations of <i>T. vulgaris</i> in Jordan						
	Northern region		Middle region		Southern region		
	Irbid	Ajlune	Jeresh	Amman	Al-Tafeileh	Shouback	Maan
Moisture (%)	6.10 ± 0.00	6.40 ± 0.0017	6.40 ± 0.00	6.50 ± 0.00	6.20 ± 0.00	6.10 ± 0.00	6.30 ± 0.00
Essential Oils(EO %)	3.30 ± 0.01	5.20 ± 0.09	5.40 ± 0.12	2.70 ± 0.003	2.30 ± 0.002	4.00 ± 0.007	2.00 ± 0.00
Mean of EO %	4.63 ± 0.07			2.70 ± 0.003	2.77 ± 0.003		

Table II. Averages of ambient temperatures (°C), rainfall (mm), and relative humidity (%) in the various climatic regions in Jordan

Place of Thyme plant cultivation	Altitude (m)	Ambient temp. Min. – Max. (°C)	Seasonal means of rainfall (mm)	Relative humidity (%)
Northern Regions				
Irbid	616	13.0 – 23.5	455.4	68.30
Ajlune	1150	10.1 – 18.6	582.2	80.20
Jeresh	540	8.5 – 18.40	445.6	68.30
Middle Region				
Amman	1050	6.8 – 23.5	490.0	51.70
South Regions				
Al-Tafeileh	1260	11.8 – 23.4	237.6	23.30
Al-Shouback	1365	4.11 – 19.9	294.2	24.80
Maan	1069	20.0 – 32.0	44.20	57.80

Table III. Concentrations of heavy metals (mg kg⁻¹) in *T. vulgaris* plant cultivated in different environmental regions in Jordan

Heavy Metal Location	Cd	Pb	Ni	Cu	Mn	Zn
Northern Region						
Irbid	ND	37.82 ± 0.06	18.31 ± 0.04	6.39 ± 0.7	2.73 ± 0.48	55.31 ± 0.36
Ajlune	ND	36.86 ± 0.14	15.85 ± 0.01	6.63 ± 0.16	25.04 ± 0.16	52.07 ± 0.09
Jeresh	ND	25.27 ± 0.04	7.840 ± 0.10	3.33 ± 0.07	26.81 ± 0.16	16.31 ± 0.25
Mean	< 1.2	33.31 ± 0.08	14.00 ± 0.05	5.45 ± 0.10	18.19 ± 0.27	41.23 ± 0.23
Middle Region						
Amman	< 1.2	22.38 ± 0.05	31.86 ± 0.05	5.31 ± 0.04	21.50 ± 0.30	45.82 ± 0.21
Southern Region						
Al-Tafeileh	ND	32.03 ± 0.08	25.70 ± 0.02	4.35 ± 0.01	3.137 ± 0.05	48.21 ± 0.23
Al-Shouback	ND	32.03 ± 0.04	23.85 ± 0.03	13.23 ± 0.13	15.52 ± 0.16	16.18 ± 0.24
Maan	ND	24.31 ± 0.05	17.08 ± 0.16	6.33 ± 0.06	23.89 ± 0.19	81.80 ± 0.25
Mean	< 1.2	29.46 ± 0.06	22.21 ± 0.07	7.97 ± 0.07	14.18 ± 0.13	48.73 ± 0.24
Detection Limit (DL)	1.2	12.0	4.0	3.0	2.8	1.1

Means ± S.D. ppm; (n = 3)

concentration of Pb is mainly attributed to motor-vehicle exhausts. Al-Alawi and Mandiwana (2007) reported a high concentration of Pb (196 mg kg⁻¹) along the roadside of Amman city was much higher than 19 mg kg⁻¹ recorded in the un-contaminated area that was 60 km away from Amman City. Moreover, Al-Khlaifat and Al-Khashmanb (2007) reported that The Pb levels were the highest (177 mg kg⁻¹) at highway sites, which have higher traffic density than background site (41 mg kg⁻¹) of Aqaba city in Jordan. On the other hand, high levels of Pb in thyme could be hazardous to human health (Macrae *et al.*, 1993).

The average values of Ni concentrations in thyme cultivated in the middle region in Amman; the capital city of Jordan country, were higher than their corresponding values in the south and north of Jordan. It ranged between 7.84 in Jeresh and 18.31 mg kg⁻¹ in Irbid with an average of 14.0 mg kg⁻¹ at the north region, then it increased to 31.86 mg kg⁻¹ in Amman, while it was decreased from 25.7 mg kg⁻¹ in Al-

Tafeileh to 17.08 mg kg⁻¹ in Maan with an average of 22.21 mg kg⁻¹ in the south region of Jordan (Table III). Ni accumulation profiles among cultivated areas of *T. vulgaris* in Jordan were Jeresh < Ajlune < Irbid at the north and Maan < Al-Shouback < Al-Tafeileh in the south region. In this study, levels of Ni in all specimens of thyme indicated higher concentrations than the average (0.1 – 0.5 mg kg⁻¹) reported by Kastori *et al.* (1993) for the same plant. This higher concentration of Ni may be due to the vicinity of road and the intensity of plant, which is progressively increased with locations full of vehicles and industrial activities in Amman or neighboring southern locations with cement factories in Al-tafelah and Al-Shoubak (Jaradat *et al.*, 1999).

Mn concentration values ranged between 2.73 in Irbid and 26.81 mg kg⁻¹ in Jeresh with an average of 18.19 mg kg⁻¹ at the north environment, while it decreased from 21.50 mg kg⁻¹ in the middle cultivated area to 18.19 and 14.18 mg kg⁻¹ at the north and south cultivated areas. The average

concentration of Mn in the middle cultivated areas was higher than northern and southern areas. Loranger and Zayed (1994) reported that, Mn and Ni are higher in industrial and residential areas, which may be due to their use as fuel additives just like Pb. Means of other metals; Cu and Zn in thyme cultivated in the south region were higher than middle and north-cultivated areas (Table III). Zn values ranged between 16.31 mg kg⁻¹ in Jeresh and 55.31 mg kg⁻¹ in Irbid with an average of 41.23 mg kg⁻¹ at the north region, while it was increased from 48.21 mg kg⁻¹ in Al-Tafeileh to 81.80 mg kg⁻¹ in Maan in the south cultivated areas. The average contents of Cu were 5.31, 5.45, 7.97 mg kg⁻¹ in the middle, north and south-cultivated areas, respectively. These results agree with those of Razic *et al.* (2003), who found that the contents of Cu in some medicinal plants in the same family varied between 5.92 and 14.79 mg/kg. However, the results of Amr and Đorđević (2000) were in the contrary who recorded 61 to 70 mg kg⁻¹ contents of Cu in *S. officinalis* specimens.

The presence of Zn, Cu and Mn in plants may be correlated with therapeutic properties against diabetic and cardiovascular diseases (Perry, 1972; Parman *et al.*, 1993). Zn has an important role in the metabolism of cholesterol as well as heart diseases (Hooker, 1982). Deficiency or excess of Cu, Mn and Zn and other essential trace elements may cause a number of disorders (Ahmed *et al.*, 1994). These elements also take part in neurochemical transmission and also serve as constituent of biological molecules, as a cofactor for various enzymes and in variety of different metabolic processes (Mayer & Vyklicky, 1989). On the other hand, WHO limits for Ni, Zn, Cu and Mn metals have not yet been established. According to Bowen (1966) and Allaway (1968) the range of the elements in agricultural products should be between 4-15 mg kg⁻¹ for Cu and 15-200 mg kg⁻¹ for Zn. It was found that Zn and Cu concentrations in all specimens were observed to be within the range among all the cultivated regions of thyme in Jordan.

CONCLUSION

The contents of essential oil in thyme are mainly affected by variable natural climatic conditions in different geographical regions are distributed between the south and north areas of Jordan country. Moreover, *T. vulgaris* grown in Jordan has high concentration of Pb element, which may has a great health hazard. Further studies of heavy metals contents in agricultural plants grown in Jordan environment are recommended.

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