J. BIOL. ENVIRON. SCI.,
2016, 10(29), 45-51

Original Research Article

Regional Altitude and Soil Physicochemical Factors Influence the Essential Oil of *Thymus pubescens* (Lamiales: Lamiaceae)

Yousef Imani Dizajeyekan, Ahmad Razban Haghighi* and Tohid Ebrahimi Gajoti
East-Azerbaijan Research Center for Agriculture & Natural Resources, 5355179854 Tabriz, IRAN

Received: 25.04.2016; Accepted: 01.08.2016; Published Online: 19.10.2016

ABSTRACT

The aim of this research was to study the effects of regional altitude and soil physicochemical factors on essential oil of *Thymus pubescens*. To achieve this goal, aerial parts of this species and top 30 cm soil samples of from five regions in East Azarbaijan province of Iran was collected. Oil samples were extracted by hydrodistillation method. Percentage of essential oil with soil pH has a significant positive relation and there is significant negative relation with altitude. Evaluating environmental factors, we found regional altitude and soil pH as the important factors influencing the production of oil. We conclude that ecological factors are important and should be taken into account for cultivation of *Thymus pubescens*.

Keywords: Correlation, Essence, Hydrodistillation, Linear regression, *Thymus pubescens*

INTRODUCTION

Thymus pubescens Boiss.&Kotschys ex Celak from Lamiaceae family is perennial herb, semi shrub, often torpid and nearly grass-green form with oval leaves and pink-white flowers (Mozaffarian 1996).Thymus essential oil has diverse applications in medicinal, cosmetic, hygienic, and food industrials.Thyme oil have properties such as antispasm, antifungal, antiseptic,carminative, and spectorant(Jahanara and Haerizadeh 2001). This reagents thymol and carvacrol give the antifungal,antibacterial, and anti-oxidant effects for the oil(Momeni and Shahroki 1998).It has been revealed that altitude has significant positive effect on the quality and quantity of essential oils of *Thymus fallax* in Lorestan natural habitats (Mohammadian *et al.* 2015).According to a study conducted on *Thymus serpyllum*,altitude in most areas has a negative impact on the quantity of oil (Abu Darwish *et al.* 2009).In agreement, Habibi *et al.*(2007) also reported a negative correlation between the altitude and the quantity of essential oil in wild thyme oil (*Thymus kotschyanus*) grown in Taleghan.Takaloo *et al.* (2012) studied the composition of the oil from *Thynus migricus* and showed that the highest yields were obtained in the flowering stage and at the lowest altitude.However,evaluating the effect of environmental factors on essential oil of *Thymus kotschyanus* in Iran found the altitude with a positive effect on the amount of essential oil while soil pH had a negative effect on the oil quantity of this species (Aminzade *et al.* 2010). Additionally, study on the Thymus species oils at South Africa's various soils and climates showed that the only *Thymus gugonii* extract has an inverse correlation with altitude.Type and percentage of oil compounds change by environmental fluctuations (Ghorab *et al.* 2013).The results of study on *Thymus kotschyanus* oil showed that the altitude, organic materials, and the soil electrical conductivity has direct effect on essences content while lime and nitrogen content have an inverse relationship with oil yield (Aminzade *et al.* 2010).Study on qualitative changes of *Thymus praecox* essence in the south of Turkey revealed a negative correlation between altitude and essence quantity and found the quality of essential oil dependent on the environmental conditions (Avci 2011;Alizadeh *et al.* 2011).The results of a study (Alizadeh *et al.* 2011) on *Thymus vulgaris* showed that the oil yield is directly related to altitude. *Thymus vulgaris* essence study in Jordan indicated that altitude, humidity and temperature have a positive effect on oil yield (Hudaib and Aburjai 2007). Tabrizi *et al.* (2010) showed that the amount of oil in *Thymus transcaspicus* is correlated with the altitude and the quality of the oil depends on the region conditions. *Thymus polegioides* L. chemotypes pattern changes study in Slovakia has shown that these patterns are related to soil factors (Martonfi *et al.* 1994). Looking at the effects of environment factors on *Thymus piperella* essence he shows that climate factors are less effective than soil factors on quantity and quality oil (Boira and Blanquer 1998). The chemical varieties of essential oil in the identified five chemotypes of *Thymus*

* Corresponding author: a_razban_h@yahoo.com

algeriensis from north Africa are mostly depend on population sites (Eight wild populations of *T. algeriensis* from different regions) (Zouari *et al.* 2012). Study on *Thymus transcaspicus* demonstrate essence quality related to regional conditions and soil type (Tabrizi *et al.* 2010). Different studies on *Thymus vulgaris* show that quality and quantity of essence related to soil parameters (nitrogen, phosphorous and potassium fertilizers (Sharafzade *et al.* 2011). Soil nitrogen and altitude have also been found important factors with reverse effects on Achillea oil (Azarnivand *et al.* 2010). Growth and proceeds of plant in various ecosystems depend on different factors such as climate, soil, and geography coordinates (Omid beigi 1995). Every of this factors known to play their own roles on quality and quantity of plants oil hence present work was performed. Therefore, it was very important to study the relationship between regional altitude and soil physicochemical factors with essential oil of *Thymus pubescens* in order to find suitable ecological conditions for plant domestication and improved production of oil.

MATERIALS AND METHODS

Flower heads collection

Sampling from flower heads of *Thymus pubescens* were collected from five region in natural habitats of East Azarbaijan in 2010 (Table 1 and Figure 1) (Imani *et al.*, 2015)

Table 1. Specifications of study areas.

Region	Longtitude	Latitude	Altitude(m)
Mishoo	45° 41′ 10″	38° 23′ 64″	2000
Yuzband	47° 07′ 31.9″	38° 44′ 48.7″	1700
Egdelo	46° 18′ 55.4″	37° 09′ 00.5″	1600
Kordedeh	46° 26′ 18.1″	37° 31′ 30.3″	2100
Sahand	46° 30′ 55″	37° 47′ 25″	2800

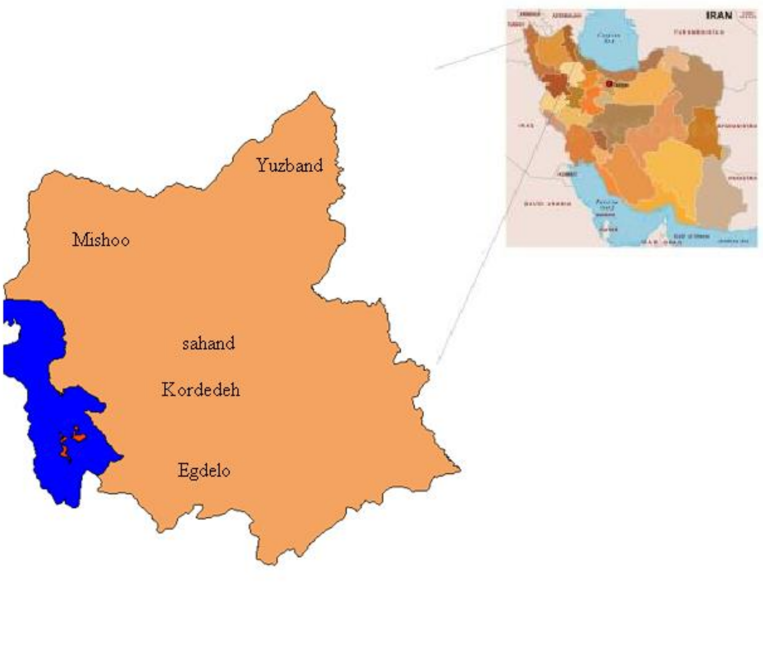

Figure 1. Study areas for *Thymus pubescens* of East Azerbaijan province in Iran map.

Essential oils extraction and Gas Chromatography (GC) analysis

Samples were dried in shade and at room temperature and essential oils were extracted by the hydrodistillation method using Celevenger apparatus during 2.5 hours. Essences were dried by anhydrous sodium sulphate and dry weight measured (Clevenger 1928). Analysis of essential oil samples using GC and GC / MS were performed and main compounds (Table 2) were identified (see Shibamoto 1987; Adams 1997; Hudaib and Aburjai 2007).

Table 2. Essential Oil Content (major compounds) in *Thymus pubescens.*

Code No	Compound	RI=retention index	Sahand	Kordedeh	Egdelo	Yuzband	Mishoo
1	p-cymene	1054	4.3	2.9	7.2	1.6	4.1
2	γ-terpinene	1082	3.4	3.3	2.2	6.5	6.2
3	α-terpineol	1234	0	4.8	0.6	6.8	0.2
4	thymol	1321	30.9	27.6	65.6	57.8	1.7
5	carvacrol	1331	3.1	25.4	3.7	4.4	82.1
6	Essential Oil (%)	-	0.31	0.55	1.34	1.26	1.16

Soil collection and analysis

Top 30 cm soil samples were collected,blended and analyzed.In order to determine the percentage of sand,clay and silt,the soil samples in were air-dried and passed from two millimeters sieve.(Table 3).Soil pH was determined following a 1:1 soil to water dilution step (Thomas 1996). Electrical conductivity (EC) was determined by a conductivity meter. Total nitrogen (N) was determined using the Kjeldahl procedure (Bremner and Mulvaney 1982). Phosphorus (P) was measured by Olsen method.(Moreno *et al.* 2007).potassium(K) were measured using a flame photometer (Richards 1954). Total neutralizing value (TNV) was determined by neutralization with HCl (Loeppert and Suarez 1996). Organic matter was determined by titration method (see Walkley and Black 1934).

Table 3. Specifications of Soil physicochemical factors for the study areas.

Location	EC ms/cm	pH	T.N.V %	OC %	P(ava) ppm	N %	K(ava) ppm	Sand%	Silt %	Clay %
Mishoo	0.68	7.91	38	1.57	4.2	0.16	273	62	22	16
Kordedeh	0.63	6.9	1	1.41	11.2	0.15	291	64	20	16
Egdelo	0.59	7.53	15	1.16	31.2	0.12	667	48	28	24
Yuzband	0.8	7.69	14.5	2.25	6	0.23	364	60	24	16
Sahand	0.74	6.23	1	4.68	11.6	0.45	510	52	26	22

*:pH= potential of Hydrogen,EC= Electrical Conductivity ,N= Total Nitrogen,P= Phosphorus,K= Potassium,TNV = Total Neutralizing value,O.C= Organic Carbon

Data Analysis

Pearson correlation coefficient and regression equations based on ten soil physicochemical factors along with regional altitude and six essential oil compounds were done using XLSTAT 2010 software (Addinsoft 2010).

RESULTS

The main compounds from interpretation of spectra by GC and GC / Mass essential oil samples to identify, thymol (1.7 - 65.6), carvacrol (3.1 - 82.1), γ-terpinene (2.2 -6.5), p-cymen (1.6 -7.2) α- terpineol (0 - 6.8) percent.(Table2). The Pearson correlation between these factors is presented in Table 4.The simple correlation coefficient among these factors indicated that significant positive correlations were between the soil pH and the essential oil percent ($r = 0.929$) while there was a significant negative correlation between the altitude and the essential oil percent ($r = –0.909$) (Imani *et al*. 2015). The results of the linear regression between variables are shown in figures 2 and 3, respectively. The percentage of essential oil was considered as a dependent variable in the analyses.The coefficient of determination and linear equation in any figure was come separately. The regression results in figure 1-2 show that regression slope for pH and essential oil is a significant positive slope ($b = 0.629$) in the event that regression slope for altitude and essential oil is a significant negative slope ($b = –0.001$).

Table 4. Pearson correlation coefficients among ecological factors with main compounds of *Thymus pubescens.*

Variables	EO(%)	ρ-cymen	γ-terpinen	α-terpineol	Thymol	Carvacrol	alt	EC(ms/cm)	PH	T.N.V(%)	OC(%)	P(ava) ppm	N (%)	K(ava) ppm	Sand(%)	Silt(%)	Clay(%)
EO(%)	**1**																
ρ-cymen	0.161	**1**															
γ-terpinen	0.356	-0.685	**1**														
α-terpineol	0.130	-0.785	0.356	**1**													
thymol	0.376	0.221	-0.331	0.341	**1**												
carvacrol	0.175	-0.099	0.483	-0.293	-0.841	**1**											
alt	**-0.909**	-0.063	-0.172	-0.384	-0.485	-0.039	**1**										
EC(ms/cm)	-0.098	-0.693	0.705	0.405	0.004	-0.161	0.261	**1**									
PH	**0.929**	-0.051	0.553	0.181	0.049	0.483	-0.848	-0.070	**1**								
(%)T.N.V	0.681	0.085	0.606	-0.281	-0.392	0.788	-0.428	-0.031	0.825	**1**							
OC(%)	-0.673	-0.103	-0.014	-0.240	-0.097	-0.355	0.849	0.595	-0.748	-0.438	**1**						
P(ava)ppm	0.235	0.842	-0.807	-0.339	0.646	-0.491	-0.317	-0.682	-0.059	-0.238	-0.255	**1**					
N(%)	-0.676	-0.127	-0.002	-0.214	-0.094	-0.361	0.846	0.610	-0.747	-0.447	**1.000**	-0.271	**1**				
K(ava)ppm	0.137	0.799	-0.664	-0.433	0.691	-0.643	-0.074	-0.307	-0.218	-0.279	0.165	**0.879**	0.149	**1**			
(%)sand	-0.087	-0.807	0.590	0.552	-0.578	0.567	-0.049	0.222	0.253	0.208	-0.293	-0.792	-0.275	**-0.981**	**1**		
silt(%)	0.249	0.691	-0.383	-0.439	0.649	-0.570	-0.067	-0.038	-0.097	-0.094	0.287	0.697	0.272	**0.941**	**-0.967**	**1**	
clay(%)	-0.049	0.861	-0.730	-0.616	0.491	-0.537	0.141	-0.360	-0.366	-0.291	0.284	0.831	0.265	**0.965**	**-0.978**	**0.892**	**1**

Values in bold are statistically different from 0 ($\alpha < 0.05$)

* pH= potential of Hydrogen,EC= Electrical Conductivity ,N= Total Nitrogen,P= Phosphorus,K= Potassium,TNV = Total Neutralizing value,O.C= Organic Carbon,alt=altitude,E.O= Essential Oil

DISCUSSION

According to the results (high coefficient of determination for linear equations), altitude has a negative effect on the percentage of essential oils (Figure 2). This is in agreement with the results of some previous works on the essential oil of different species of the this genus (Habibi *et al.* 2007; Takaloo *et al.* 2012).This means that with increasing altitude, plant growth and essential oil production will be limited. The effect of soil pH (6-8) on essential oil content showed that the mean percentage of essential oil increases by increasing soil pH (Figure 3). It seems that in this area by increasing the pH levels, the activity of soil biological factors such as microorganisms and earthworms will increase. This activity by the soil minerals and nutrients are dissolved and accelerates the active ingredients produce in plant. The results of this study confirm this subject (Aminzade *et al.* 2010). The effect of environmental factors on secondary plant metabolites is important and many studies have ever been done about it (Avci 2011). Plants active ingredients under the effects of these factors vary in terms of quality and quantity. The results of the present study focused on the effects of ecological factors introduce the altitude and soil pH as the influencing factors of the quantity of essential oil in *Thymus pubescens.* These results indicate that environmental factors could have an effect on the quality of the oil, which corresponds with the results of previous work on this genus (Boira and Blanquer 1998; Omid beigi 1995). Based on the results of this study, it was found that essential oil of *Thymus pubescens* changes under the influence of environmental factors.The range of soil pH (6-8) has a positive effect on oil yields. To improve the production of the essence of the plant species, it is recommended that this species to be cultivated in areas with the slightly acidity to slightly alkaline soils and low altitude.

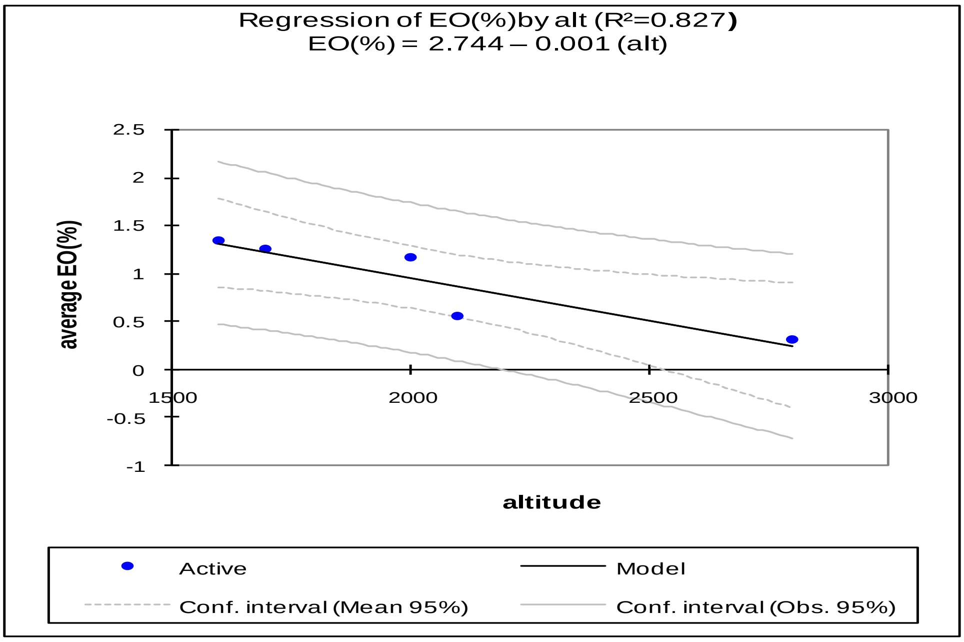

Figure 2. The effect of areas altitude on average essential oil (%) Essential Oil (%) = 2.744 – 0.001 (altitude) (R^2= Coefficient of Determination = 0.827).

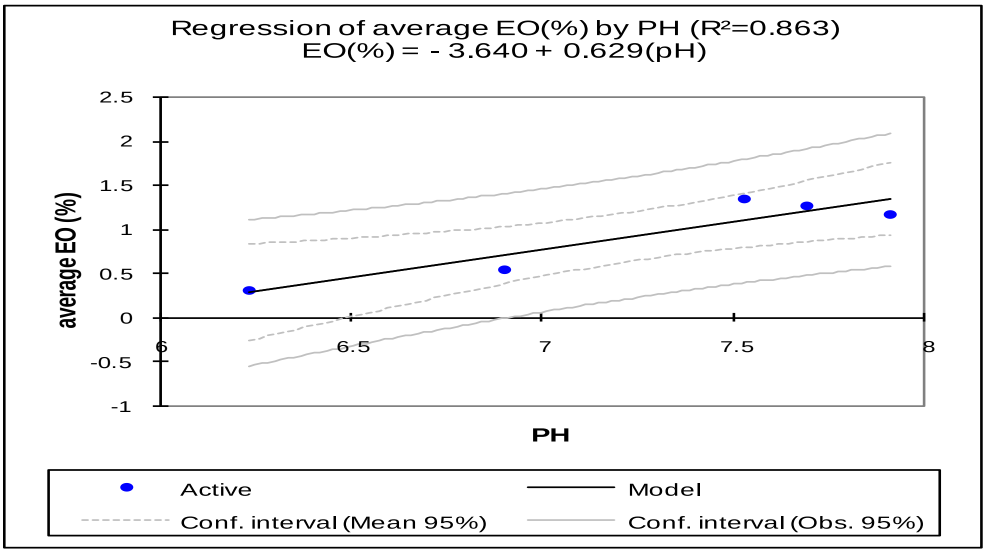

Figure 3. The effect of areas soil PH on average essential oil (%) Essential Oil (%) = -3.640 + 0.629 (PH= potential of Hydrogen) (R^2= Coefficient of Determination = 0.863).

REFERENCES

Abu Darwish MS ,Abu Dieyeh ZH, Mufeed B, Al Tawaha ARM and Al Dalain SYA(2009).Trace element contents and essential oil yields from wild thyme plant (*Thymus serpyllum* L.) grown at different natural variable environments, Jordan. J Food Agric Environ.7:920-924.

Adams RP (1997).Identification of Essential Oil Components by Gas chromatography/Mass Spectroscopy. J.Am. Soc. Mass Spectrom. 6(8) :671-672.

Adinsoft SARL(2010). XLSTAT.software, version 10. Addinsoft, Paris, France.

Alizadeh A, Alizadeh O, Sharafzadeh SH and Mansoori S (2011).Effect of different ecological environments on growth and active substances of garden thyme. Adv EnvirBio. 5 : 780-783.

Aminzadeh M.,Amiri F.,Abadi E.A.,Mahdevi K. and Fadai S.(2010). Factors Affecting on Essential Chemical Composition of *Thymus kotschyanus* in Iran.World Appl Sci J. 8(7) :847.856.

Avci AB (2011).Chemical variation on the essential oil of *Thymus praecox* ssp. *scorpilii* var. Laniger. Int J Agric Biol.13:607.610

Azarnivand H,Ghavam AM,Sefidkon F and Tavili A (2010).The effect of ecological characteristics on quality and quantity of the essential oils of *Achillea millefolium* L. subsp. *millefolium*.Iran J Med &Arom plants.25(4): 556-571

Boira H and Blanquer A (1998).Environmental factors affecting chemical variability of essential oils in *Thymus piperella* L.Biochem Sys Ecol.26(8):811-822.

Bremner JM and Mulvaney CS (1982).Nitrogen.total. Part II.Chemical and Microbiological Properties.In: Miller A. L., R. H. and Keeney D. R. (eds.): Methods of Soil Analysis, Second Edition, Am. Soc. Agron, WI, B.USA, PP. 595–624

Clevenger JF(1928).Apparatus for the determination of volatile oil.J Am Pharmaceut Assoc.17(4):345-349.

Ghorab H,Kabouche A and Kabouche Z (2014).Comparative compositions of essential oils of Thymus growing in various soils and climates of North Africa.Sahara Environ Sci. 5 (1) :298-303

Habibi H, Mazaheri D, Majnoon Hosseini N,Chaeechi MR, Fakhr Tabatabaee M and Bigdeli M (2007). Effect of altitude on essential oil and components in wild thyme (*Thymus kotschyanus* Boiss) Taleghan region. Agro J (Pajouhesh & Sazandegi).73:2-10

Hudaib M and Aburjai T (2007).Volatile components of Thymus vulgaris L.from wild-growing and cultivated plants in Jordan. Flavour Frag. J. 22(4):322-327.

Imani Y, Razban Haghighi A, Sefidkon F and Naderi M (2015). Investigation on chemotypes of Thymus pubescens Boiss.et Kotschy ex Celak based on essential oil compounds in E-Azerbayjan province. Iranian J Med Aromat Plants.,31(3):396-405

Jahanara F, and Haerizadeh B (2001).Information and application of Iranian official herbal drugs. Razi darou gostar pub.208 p.

Loeppert R.H., Suarez D.L.1996.Carbonate and gypsum.In: Sparks D.L.(eds) Methods of Soil Analysis. Part III. 3rd Ed. Am. Soc. Agron., Madison,WI. pp. 437.474.

Mártonfi, P, Grejtovský, A and Repčák, M (1994).Chemotype pattern differentiation of *Thymus pulegioides* on different substrates.Biochem Syst Ecol. 22(8):819-825.

Mohammadian A , Karamian R, Mirza M, Sepahvand A (2015).Effects of altitude and soil charactristics on essential oil of *Thymus fallax* Fisch. Et C.A. Mey. in different habitats of Lorestan province. Iran J med & arom plants. 30(4): 519-528.

Momeni TK And Shahroki NB (1998).Vegetable oils and their therapeutic effects, Tehran university press, Iran.171p.

Moreno G ,Obrador JJ and García A (2007).Impact of evergreen oaks on soil fertility and crop production in intercropped dehesas. Agric Ecosyst Environ.119(3):270-280.

Mozaffarian VA (1996).Dictionnary of Iranian plant Names.Farhang Moaser, Pub,Tehran.Iran. 740 p.

Omid Beigi R(1995).Propagation and Production of Medicinal plants.Vo l1.Fekrafroz Pub.Tehran.Iran.,283 p.

Richards LA (1954).Diagnosis and improvement of saline and alkali soils.Soil Science. 60.U.S. Dept Agric. Handbook,154p.

Sharafzade S, Alizadeh O and Vakili M(2011).Effecet of Nitrogen Sources and Levels on Essential Oil Components of *Thymus vulgaris* L. Australian Journal of Basic and Aplied Sci, 5(10): 885-889.

Shibamoto T (1987).Retention indices in essential oil analysis in: Sandra P., Bicchi C.(eds) Capillary Gas Chromatomatography in Essential Oils Analysis.Dr. Alperd Huethig Verlag, New York.pp.259.277

Tabrizi L,Koocheki A,Moghaddam PR and Mahallati MN(2010).Chemical composition of the essential oils from *Thymus transcaspicus* in natural habitats.Chem Nat Compd.46(1):121-124.

Takaloo SG,Hassani A,Hassanpouraghdam MB,Meshkatalsadat MH,Pirzad A and Heidari M(2012).Essential Oil Content and Composition of Thymus migricus Klokov & Desj-Shost. Affected by Plant Growth Stage and Wild Habitat Altitude.Rom Biotech Lett.17(1):6982-6988.

Thomas GW(1996). Soil pH and soil acidity. In: Sparks, D.L. (Ed.) Methods of Soil Analysis, Part 3, Chemical Methods. SSSA Book Series No. 5., 677 S. Segoe Rd., Madison, WI 53711, pp. 475-490.

Walkley A and Black IA(1934).An examination of the Degtjareff method for determining soil organic matter ,and a proposed modification of the chromic acid titration method. Soil Sci. 37(1): 29-38.

Zouari N, Ayadi I, Fakhfakh N, Rebai A, and Zouari S (2012).Variation of chemical composition of essential oils in wild populations of *Thymus algeriensis* Boiss. et Reut., a North African endemic Species. Lipids in Health Dis., 11(1):11-28.