The Effects of Urea on Essential Oil Composition of *Thymus vulgaris* Linn.

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Abstract

Thyme or *Thymus vulgaris* Linn. (*T. vulgaris*) belongs to family Lamiaceae. It is considered the principal species for its uses, fresh and dried as a culinary herb. Its essential oil (EO) also employed in perfumery and in food industry. The EO possesses antiseptic and antifungal properties and is used for medicinal purposes. Urea is one of the major nitrogen (N) forms supplied as fertilizer in to agriculture crops. The effects of urea (0, 125, 250, 375 and 500 kg ha^{-1}) on the EO composition were investigated. The highest EO contents (0.5 % and 0.6 ml $Plant^{-1}$) were recorded with 375 kg $ha^{-1}$of urea. Twenty components were identified by GC and GC/ MS in the EO of each treatment; thymol, γ-terpinene and p-cymene were detected as the major components which increased with different urea doses. Monoterpenes were the major classes of EO isolated from *T. vulgaris* herb. The highest values of major components (43, 23.6 and 17.6%) were recorded with treatment of 250 kg $ha^{-1}$of urea. The highest values of monoterpene hydrocarbons (MCH) and oxygenated sesquiterpenes (SCHO) (53.6 and 1.3%) were recorded with 375 kg $ha^{-1}$of urea. The highest contents of oxygenated monoterpenes (MCHO) (53.6%) and sesquiterpene hydrocarbons (SCH) (0.6%) were recorded with treatments of 250 and 125 kg ha^{-1} of urea respectively.

Keywords: thyme, urea, essential oil, thymol, monoterpenes, sesquiterpenes

Introduction

The genus *Thymus* includes numerous species with very different botanical characteristics and a large chemical heterogeneity that have achieved economic importance (Stahl-Biskup, 1991). *T. vulgaris* belongs to family Labiatae or Lamiaceae. It is considered the principal species for its uses, fresh and dried as a culinary herb. *T. vulgaris* EO also employed in perfumery and in the food industry. The EO possesses antiseptic and antifungal properties and is used for medicinal purposes (Aureli et al., 1992; Hammer et al., 1999; Dorman and Deans, 2000; Bisset, 2001). Pharmacological action of thyme, due mainly to the presence of phenolic compounds (Bisset, 2001; Yanay, 2007) is strictly dependent on the EO content; differences in the chemical composition arise from the existence of different chemo types (De Bouchberg et al., 1976; Adzet et al., 1977; Rhyu, 1979; Lemordant, 1986).

The EO is originated in the secondary metabolism of the plant; however, the compounds of this pathway depend on the primary metabolism. Thus, plant productivity, which reflects the primary metabolism and it is determined by biomass, depends on leaf quantity, photosynthetic capacity of each leaf and nutrient availability. The suitable supply of mineral nutrients for the culture is one of the most important factors to improve EO productivity (Fageria et al., 1977).

Urea is one of the major nitrogen (N) forms supplied as fertilizer in agriculture, but it is also an important N metabolite in plants. The interest in urea fertilizers due to the rapid and efficient response to the plant needs. It is also recognized that supplementary urea application during plant growth can increase the EO of aromatic plants

(Watson et al., 1974). The p-cymene, γ-terpinene and carvacrol components of thyme EO increased under urea treatments, while thymol content decreased (Sharafzadeh et al., 2011). Urea caused a significant increase in lemon balm EO and its major constituents (Aziz and El-Ashry, 2009). The urea doses did not significantly affect the EO yield of *M entha piperita* L. but menthone levels were changed (Deschamps et al., 2012). Urea application had a significant effect on EO percentage and EO composition of *Melissa officinalis* L. (Abbaszadeh et al., 2009).

Therefore, an attempt was made to follow the effects of urea on EO composition isolated from *T. vulgaris* an important aromatic plant.

Materials and Methods

Site, Plan and Methodology

Experiments were carried out in sandy soil at the Experimental Farm of National Research Centre (NRC) in Nubaria region, Egypt, during the season of 2013. Seedlings of *T. vulgaris*, which were provided by the Department of Medicinal and Aromatic Plants, Ministry of Agriculture, Giza, Egypt, and transplanted into the open field. The experimental design was a complete randomized block with four replicates. The experimental area (plot) was 4 m² containing 4 rows (each plot contained 32 plants); the distance between hills was 25cm and 50cm apart. Thinning for two plants per hill was made 45 days after transplanting. All cultural practices operations other than experimental treatments were performed according to the recommendations of the Ministry of Agriculture, Egypt. Plots were divided into five main groups. The first, second, third and fourth groups subjected to soil application of urea (46% N) at 125, 250, 375 and 500 kg ha^{-1} respectively. The plants of 5th group were kept as control. Doses were selected hypothesized, as per citation of literature for optimum results consideration.

Harvesting

At full bloom stage, the plants were harvested by cutting the plants 5 cm above the soil surface.Total mass productions (fresh and dry weights) $gram^{-p}$ were recorded accordingly.

EO Isolation

Fresh herbs were collected from each treatment, while 300g from each replicate of all treatments was subjected to hydro-distillation for 3 h using a Clevenger type apparatus (Clevenger, 1928). For distillation, a mixture of fresh herbs (300 g) and 1000 ml of water was put into a 2000 ml round bottomed flask. The temperature was set at 100 °C for the extraction of essential oil. The process in Clevenger-type apparatus was run till such time that no further oil could be extracted. The EO was vaporized with steam. Condensation occurred as the vapors of EO and steam mixture passed through a condenser. The condensate, a mixture of EO and water, was then separated. EO being lighter settled above water was collected. The EO content was calculated in percentage. In addition, total EO per plant was calculated by using the dry weight of the herbs.

GC and GC–MS Conditions

GC analyses were performed using a Shimadzu GC-9 gas chromatograph equipped with a DB-5 (dimethyl siloxane, 5% phenyl) fused silica column (J & W Scientific Corporation) (30 m, 0.25 mm i.d., film thickness 0.25 lm). Oven temperature held at 50°C for 5 m and then programmed to rise to 240°C at a rate of 3°C min^{-1}. The flame ionization detector (FID) temperature was 265°C and injector temperature was 250°C. Helium used as carrier gas with a linear velocity of 32 cm s^{-1}. The percentages of compounds were calculated by the area normalization method, without considering response factors.

GC–MS analyses were carried out in a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m, 0.25 mm i. d., film thickness 0.25 lm; (oven temperature was 50-240°C at a rate of 4°C min^{-1}, transfer line temperature 260°C, carrier gas, helium, with a linear velocity of 31.5 cm s^{-1}, split ratio 1:60, ionization energy 70 eV, scan time 1 s, and mass range 40-300 amu.

Identification of EO Components

The components of the oils were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices, either with those of authentic compounds or with

data already published (Adams, 1995). Mass spectra from the literature were also compared (Adams, 1995). The retention indices were calculated for all volatile constituents using a homologous series of n-alkanes.

Statistical Analysis

In this vary experiment, one factor was considered: urea doses in form of N (0, 125, 250, 375 and 500 kg ha^{-1}) was applied. For each treatment there were 4 replicates. The experimental design followed a complete randomized block design. Average Data were statistically analyzed using 1-way analysis of variance (ANOVA-1) (Snedecor and Cochran, 1990). Significant values determined according to P values ($P<0.05$ = significant, $P<0.01$ = moderate significant and $P<0.001$ = highly significant). The applications of that technique were according to the STAT-ITCF program (Foucart, 1982).

Results

Effect of Urea Doses on EO Content

The contents of EO (% and mL $plant^{-1}$) increased with an increase of different doses of urea. Highest contents of EO were recorded with 375 kg ha^{-1} of urea with the values of 0.5% and 0.6 mL $plant^{-1}$ (Table 1). The lowest values EO contents (0.3 % and 0.1 mL $plant^{-1}$) were recorded with control (0.0 kg ha^{-1}). ANOVA indicated that EO percentage was non-significant while EO yields were significant in various urea doses.

Effect of Urea Doses on EO Constituents

The GC–MS analysis revealed the presence of twenty different compounds identified (Table 2). In this vary study; thymol, γ-terpinene and p-cymene were detected as the major compounds which gave the highest percentages (> 75%) of the EO in all urea doses that increased under different urea doses (Table 2). Constituents were identified in EO extracted from *T. vulgaris* herb belong to four chemical classes. The class of monoterpene hydrocarbons (MCH) was the major one, followed by oxygenated monoterpenes (MCHO). The remaining fractions as, sesquiterpene hydrocarbons (SCH) and oxygenated sesquiterpenes (SCHO) formed the minor classes (Table 2). The highest values of major components [thymol (43%), γ-terpinene (23.1%) and p-cymene (17.6%)] resulted from the 250 kg ha^{-1} of urea compared with other urea doses. MCH increased with various doses of urea compared with control while MCHO, SCH and SCHO changed (increased & decreased) or stabilized.

Table 1 Effect of urea on essential oil content

Urea doses (kg ha^{-1})	Essential oil contents			
	(%)		(mL $plant^{-1}$)	
	Mean	SD	Mean	SD
0	0.3	±0.1	0.1	±0.0
125	0.4	±0.2	0.2	±0.1
250	0.4	±0.2	0.2	±0.1
375	0.5	±0.1	0.6	±0.2
500	0.4	±0.2	0.5	±0.2
F value	0.4 NS		4.1*	

The highest amounts of MCH, SCH and SCHO obtained from the treatment of 375 kg ha^{-1} of urea with the values of 53.6, 1.5 and 1.3% respectively. The highest amount of MCHO resulted from the treatments of 0.0, 250 and 500 kg ha^{-1} of urea. ANOVA indicated that the changes in camphene, β-pinene, myrcene, α-phellandrene, γ-terpinene, camphor, carvacrol, β-humulene and MCHO were highly significant for urea doses. The changes in limonene, β-caryophyllene and SCH were moderate significant. The changes in α-terpinene, linalool, borneol, α-terpineol and thymol were significant. The changes in α-pinene, sabinene, p-cymene, α-copaene, β-bourbonene, MCH and MCHO were non-significant.

Discussion

The variations in EO content and composition may be due to its effect of different urea levels on enzymes activity and metabolism improvements (Burbott and Loomis, 1969; Gobbo-Neto and Lopez, 2007). Urea, one of the major nitrogen (N) forms (Watson et al., 1974). The obtained results are at par with those resulted by Khalid, 1996 who concluded that N in form of Urea increased the EO of some Apiaceae plants. Nitrogen application

Table 2 Effect of urea on essential oil constituents.

No.	Components (%)	RI[s]	RI	Class	Urea doses (kg ha^{-1})										F value
					0		125		250		375		500		
					Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
1	.α-Pinene	939	939	MCH	1.5	±0.5	1.9	±0.4	1.0	±0.0	1.3	±0.4	1.2	±0.2	3.3 NS
2	.Camphene	952	953	MCH	2.2	±0.2	1.2	±0.2	0.3	±0.1	1.2	±0.4	2.1	±0.1	64.8***
3	.Sabinene	977	976	MCH	0.2	±0.1	0.1	±0.0	0.1	±0.0	1.1	±0.5	0.2	±0.1	2.3 NS
4	.β-Pinene	081	980	MCH	0.3	±0.1	0.1	±0.0	0.1	±0.0	0.4	±0.3	0.2	±0.1	8.5***
5	.Myrcene	990	991	MCH	1.2	±0.2	1.1	±0.1	0.2	±0.1	1.2	±0.1	1.1	±0.5	25.0***
6	.α-Phellandrene	1005	1005	MCH	1.3	±0.3	1.2	±0.2	0.2	±0.1	1.4	±0.3	1.5	±0.4	7.6***
7	.α-Terpinene	1019	1018	MCH	3.3	±0.3	3.9	±0.4	4.4	±0.4	3.9	±0.2	3.4	±0.3	4.0*
8	.p-Cymene	1027	1026	MCH	15.6	±0.2	16.9	±0.4	17.6	±0.2	16.5	±0.1	16.3	±0.1	0.3 NS
9	.Limonene	1030	1031	MCH	4.2	±0.2	4.6	±0.6	5.2	±0.2	4.3	±0.3	4.1	±0.4	5.5**
10	.γ-Terpinene	1062	1062	MCH	19.7	±0.2	21.1	±0.1	23.1	±0.1	21.0	±0.1	20.4	±0.1	19.8***
11	.Linalool	1098	1098	MCH	2.3	±0.3	1.4	±0.4	1.0	±0.0	1.3	±0.1	2.0	±0.1	3.2*
12	.Camphor	1142	1143	MCHO	2.6	±0.2	1.1	±0.1	0.6	±0.1	0.6	±0.1	1.1	±0.2	92.1***
13	.Borneol.	1166	1165	MCHO	0.2	±0.1	0.3	±0.1	0.1	±0.0	0.3	±0.2	0.4	±0.5	2.8*
14	.α-Terpineol	1190	1189	MCHO	0.6	±0.1	1.6	±0.6	1.4	±0.4	1.3	±0.3	1.5	±0.1	2.7*
15	.Thymol	1291	1290	MCHO	39.9	±0.2	41.2	±0.2	43.0	±0.3	40.7	±0.1	40.2	±0.1	1.2*
16	.Carvacrol	1298	1298	MCHO	2.0	±0.2	0.1	±0.0	0.2	±0.1	0.2	±0.1	2.1	±0.0	25.5***
17	.α-Copaene	1277	1376	SCH	0.1	±0.0	0.2	±0.1	0.3	±0.1	0.3	±0.1	0.1	±0.1	5.0 NS
18	.β-Bourbonene	1395	1394	SCH	0.2	±0.1	0.3	±0.1	0.2	±0.1	0.5	±0.2	0.2	±0.1	3.2 NS
19	β-Caryophyllene	1419	1418	SCH	0.2	±0.1	0.1	±0.0	0.2	±0.1	0.7	±0.2	0.2	±0.2	12.2**
20	.β-Humulene	1440	1440	SCHO	0.2	±0.1	0.2	±0.1	0.2	±0.1	1.3	±0.3	1.2	±0.1	31.1***
	MCH				51.8	±0.2	53.5	±0.3	53.2	±0.3	53.6	±0.2	52.5	±0.5	0.3 NS
	MCHO				45.3	±0.5	44.3	±0.4	45.3	±0.5	43.1	±0.1	45.3	±0.5	0.2 NS
	SCH				0.5	±0.1	0.6	±0.2	0.7	±0.2	1.5	±0.5	0.5	±0.1	7.6**
	SCHO				0.2	±0.1	0.2	±0.1	0.2	±0.1	1.3	±0.3	1.2	±0.2	31.1***
	Total identified				97.8		98.6		99.4		99.5		99.5		

Notes: RI, retention indices in elution order from DB-5 column; RIs, Standard RI from Adams (1995). MCH: Monoterpene hydrocarbons, MCHO: Oxygenated monoterpene, SCH: Sesquiterpene hydrocarbons, SCHO: Oxygenated sesquiterpene

increased the amount of *Ocimum basilicum* L. EO (Arabaci, and Bayram, 2004). Hellal et al., (2011) reported that N fertilizer increased the EO yield of dill (*Anethum graveolens* L.). Sarab et al. (2008) indicated that application of N caused a significant increase in EO content of basil. Kandil et al. (2009) observed that the highest basil EO yield when the N was applied. Significant affected on linalool, and germacrene D and chemical composition of the basil EO (Özcan and Chalchat, 2002; Nurzyńska-Wierdak, 2007; Nurzynska-Wierdak et al., 2013; Chang et al., 2008). The increase in N doses resulted in increase accumulation of EO and major constituent's concentration of anise, coriander and sweet fennel fruits (Khalid, 2014). Different changes were found in EO composition of Black cumin, sweet basil and some medicinal *Apiaceae* plants (Khalid, 2012; Khalid, 2015a,b,c; Mohamed et al., 2016; Khalid and Shedeed, 2015). These results showed that the forms of N like urea for production system should be considered in the chemical characterization of the EO produced from EO bearing plants when treating with urea.

Conclusions

It has been concluded that the highest contents of EO were recorded in dose of urea @ 375 kg ha^{-1} of urea with the values of 0.5% and 0.6 ml Plant^{-1}. The highest values of major components [thymol (43%), γ-terpinene (23.1%) and p-cymene (17.6%)] resulted from the 250 kg ha^{-1} of urea doses applied so far. Groups of EO were changed with various urea levels.

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