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Effect of Nitrogen Sources and Levels on Essential Oil Components of *Thymus vulgaris* L.

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Abstract: Garden thyme (*Thymus vulgaris* L.) is a small shrubby plant with a strong and spicy taste, belonging to the Lamiaceae family. Thymol and carvacrol, which are the principal constituents of thyme oil have been reported to act as antioxidant, antimicrobial and antifungal agent. Nitrogen has an important role in the growth and active substances in medicinal plants. A greenhouse experiment was conducted to evaluate the effect of nitrogen sources and levels on essential oil components of thyme. The treatments were using 50 and 100 mg N/kg (100 and 200 kg N/ha) by using two sources of nitrogen, ammonium nitrate (33% N) and urea (46% N), and comparing them to control (without using nitrogen). Experiment was carried out using a completely randomized design (CRD) with three replications. The major components were thymol (53.70-63.63%), γ-terpinene (7.66-10.82%), *p*-cymene (6.37-8.17%), carvacrol (2.86-6.69%), terpinolene (1.85-3.15%), β-caryophyllene (1.99-2.89%), linalool (1.46-2.04%). Thymol had the maximum value (63.63%) when the plants fertilized by ammonium nitrate (50 mg N/kg).

Key words: garden thyme, thymol, carvacrol, fertilizer, GC-MS.

INTRODUCTION

Thyme (*Thymus vulgaris* L.) is a perennial plant belonging to the Lamiaceae family. The green part of thyme plant constitutes the most popular herbal medicine and spice, used in all developing countries. It is 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 (Inouye *et al*., 2001; Hernandez *et al*., 2004). Essential oil content of thyme have been reported from 0.32% (Ozguven and Tansi, 1998) to 4.9% (Carlen *et al*., 2010). Thymol and carvacrol, which are the principal constituents of thyme oil (Atti-Santos *et al*., 2004; Goodner *et al*., 2006) have been reported to act as antioxidant (Dorman *et al*., 1995; Jukic and Milos, 2005; Kulisic *et al*., 2005), antimicrobial agent (Deans and Ritchie, 1987; Prabuseenivasan *et al*., 2006), antifungal agent (Klaric *et al*, 2007) treatment for respiratory tract diseases (Inouye *et al*., 2001), wound healing, a stomachic carminative, diuretic and urinary disinfectant (Boskabady *et al*., 2006). Researchers have revealed that major volatile constituents obtained from the aerial parts of the plant are geranial, linalool, carvacrol, thymol and trans-thujan-4-ol/terpinen-4-ol (Piccaglia and Marotti, 1991; Piccaglia *et al*., 1993; Omidbaigi and Rezaei Nejad, 2000; Omidbaigi and Arjmandi, 2002; Ozcan and Chalchat., 2004). In samples of thyme were collected during the flowering period in eastern Morocco (Taforalt) in May, essential oil yield was 1.0% and camphor (38.54%), camphene (17.19%), α-pinene (9.35%), 1,8-cineole (5.44%), borneol (4.91%) and β-pinene (3.90%) were the major oil components (Imelouane *et al*., 2009). Howewer, characteristic compounds of *T.vulgaris* essential oil are thymol (44.4 – 58.1 %), *p*-cymene (9.1-28.5%), γ-terpinene (6.9 – 18.9%) and carvacrol (2.4-4.2%) (Baranauskiene *et al*., 2003; Eissa *et al*., 2005; Aziz *et al*., 2008; Ezz El-Din *et al*., 2009).

Medicinal plants like other plants take nutrients from the soil during growth. Among macroelements, nitrogen results in the largest growth and yield response in medicinal plants (Cox, 1992; Ayub *et al*., 2011). High nitrogen levels may decrease medicinal plant growth and secondary metabolite accumulation (Hornok, 1983; Laughlin, 1983; Boyle and Craker, 1991). The source of nitrogen can influence growth and active substances of medicinal plants. Some of herbs such as basil and Japanese mint showed different response for production of essential oils and oil components when fertilized with No_3-N and NH_4-N (Singh and Singh, 1978; Alder *et al*., 1989).

The degree of basil plant supply with nitrogen is a major factor regarding yielding and affects the quantity and composition of volatile oils (Politycka and Golcz, 2004; Daneshian *et al*., 2009; Biesiada, and Kus, 2010). Zhang *et al*. (1996) indicated that ammonium was unfavorable to saponin formation (a secondary metabolite) on the ginseng cell growth. Nagella and Murthy (2011) reported that nitrogen source affected withanolide-A production from cell suspension cultures of *Withania somnifera*. Alkaloid accumulation was investigated in *Catharanthus roseus* seedlings under the conditions of different nitrogen sources. Increased accumulation of

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alkaloid was found in all leaf pairs, as well as in roots of *C. roseus* of NO_3^- fed plants as compared to NH_4^+ fed plants.(Misra and Gupta, 2006). Nitrogen application depends upon crop, cultivar, soil and fertility status of soil (Ayub *et al*., 2011).

This study focuses on effect of nitrogen sources and levels on essential oil constituents of garden thyme.

MATERIALS AND METHODS

Plant Material and Experimental Conditions:

This study was conducted on a greenhouse in Shahin Shahr, State of Isfahan, Iran. The seeds were sown in the pots containing 3/5 soil and 2/5 sand (v/v) and thinned at 4-6 leaves stage to one plant per each pot. The soil of pots were tested before applying treatments and soil texture was sandy clay loam with PH=7.75, organic C=0.25%, total N=0.03%, available P=6.7 mg/kg, available K=190 mg/kg and EC=1.9 dS/m. Before sowing of the seeds and according to the soil test, the growing mixture of pots was supplied with 50 mg/kg K_2O and 100 mg/kg P_2O_5. Plants kept at 23±3/15±3°C day/night temperatures. The treatments were using 50 and 100 mg N/kg (100 and 200 kg N/ha) by using two sources of nitrogen, ammonium nitrate (33% N) and urea (46% N), and comparing them to control (without using nitrogen). Experiment was carried out using a completely randomized design (CRD) with three replications. Each replicate contained 5 pots. Plants were harvested at full bloom stage, 10 cm above the pot soil surface, and were dried at room temperature.

Essential Oil Extraction:

Isolation of essential oils was performed using hydrodistillation of 20 g sample of dried shoots using a Clevenger-type apparatus over 3 hours. The oils were dried over sodium sulphate.

Gas Chromatography (GC):

Gas Chromatography analysis was performed on an Agilent technologist model (7890A) equipped with flame ionization detector and capillary column HP-5 (30 m × 0.32 mm, 0.25 µm film thicknesses). The chromatographic conditions were as follows: The oven temperature increased from 60 to 210ºC at a rate of 3ºC/min then 210 to 240 ºC at a rate of 20ºC/min. The injector and detector temperatures were 280 and 290ºC, respectively. N_2 used as the carrier gas (1 ml/min).

Gas Chromatography-Mass spectrometry (GC-MS):

Essential oil was also analysed by Hewlett- Packard GC-MS (model 6890 series II) operating at 70e V ionization energy. Equipped with a HP-5 capillary column (phenyl methyl siloxane (30 m × 0.25 mm, 0.25 µm film thickness) with He as the carrier gas and a split ratio of 1:50. The retention indices for all the components were determined according to the Van Den Doll method using n-alkanes as standard. The compounds were identified by comparison of retention indices (RRI- AP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley and mass finder 3 libraries or with the published mass spectra.

RESULTS AND DISCUSSION

Qualitative and quantitative analyses of essential oils have been shown in Table 1. Thirty three components were identified in the oil of thyme. The major components were thymol (53.70-63.63%), γ-terpinene (7.66-10.82%), *p*-cymene (6.37-8.17%), carvacrol (2.86-6.69%), terpinolene (1.85-3.15%), β-caryophyllene (1.99-2.89%), linalool (1.46-2.04%). Urea decreased (55.65 and 53.70%) and ammonium nitrate increased (63.63 and 62.15%) the thymol when compared to control (59.89%). Urea treatment when used as 100 mg N/kg (200 kg N/ha), increased *p*-cymene, γ-terpinene and carvacrol but decreased thymol when compared to control. Researchers have shown that γ-terpinene converts to *p*-cymene then thymol synthesizes during hydroxylation of *p*-cymene [Mikio and Taeko, 1962; Nhu Trang *et al*., 2006]. This can explain why thymol had low value in 100 mg N/kg supplied by urea.

Nitrogen has an important role in essential oil biosynthesis. In addition to influence on photosynthesis and respiration for carbon skeleton production, nitrogen is a part of three important coenzymes, ATP, NADPH and Co A which have important role in terpenoid biosynthesis (Sell, 2003).

Sharafzadeh *et al*. (2010) indicated that thymol was 56.9% and 55.5% in leaf and stem oil respectively. Nutrients affect growth, essential oil and total phenolic content of thyme (Sharafzadeh, 2011). Another research showed that thymol was 51.76-58.46 in different ecological conditions (Alizadeh *et al*., 2011).

In an experiment with three years old plants, thymol was not affected by nitrogen levels and the maximum value was 38.7% (Omidbaigi and Arjmandi, 2002). Geographical conditions and the age of plants can affect active substances.

Table 1. Amounts of the chemical components of thyme oil in fertilizer treatments.

RI	Component name	Control	Urea (50 mg N/kg)	Urea (100 mg N/kg)	Ammonium nitrate (50 mg N/kg)	Ammonium nitrate (100 mg N/kg)
928	α-Thujene	0.60±0.11	0.47±0.36	0.72± 0.13	0.55±0.30	0.46±0.10
934	α-Pinene	0.43±0.06	0.50±0.06	0.51±0.08	0.42±0.22	0.34±0.04
950	Camphene	0.28±0.05	0.39±0.07	0.36±0.02	0.28±0.26	0.27±0.01
973	Sabinene	0.14 ±0.01	0.19±0.04	0.18±0.03	0.11±0.09	0.13±0.02
976	1-Octen-3-ol	1.30±0.46	1.23±0.24	1.06±0.13	1.05±0.75	1.29±0.29
978	β-Pinene	0.84±0.07	0.89±0.14	0.94±0.14	0.69±0.34	0.67±0.13
990	Myrcene	0.13±0.06	0.05±0.09	t	0.09±0.08	0.11±0.01
1002	α-Phellandrene	0.12±0.01	0.17±0.06	0.09±0.08	0.09±0.08	0.37±0.47
1015	α-Terpinene	1.04±0.07	1.13±0.14	1.22±0.15	0.89±0.46	0.92±0.14
1024	*P*-Cymene	7.79±1.13	7.54±0.23	8.17±1.10	6.37±5.14	6.95±0.96
1033	1,8-Cineol	0.43±0.17	0.71±0.47	0.76±0.23	0.20±0.17	0.52±0.12
1057	γ-Terpinene	9.06±0.50	9.48±1.55	10.82±1.46	7.66±3.26	8.36±1.60
1061	(E)-Sabinene hydrate	1.55±0.29	1.42±0.14	1.49±0.11	0.96±0.66	1.40±0.15
1087	Terpinolene	2.60±0.58	2.73±0.30	3.11±0.03	1.85±1.16	3.15±0.44
1098	Linalool	1.72±0.39	2.04±0.07	1.46±0.30	1.51±0.32	1.71±0.53
1143	Camphor	0.44±0.39	0.51±0.19	0.51±0.10	0.36±0.16	0.30±0.08
1161	Borneol	0.15±0.06	0.22±0.15	0.13±0.11	0.08±0.07	0.17±0.01
1237	Thymol methyl ether	0.73±0.31	1.75±0.83	1.15±1.07	1.25±0.95	0.83±0.54
1241	Carvacrol methyl ether	0.57±0.20	0.84±0.35	0.69±0.37	0.62±0.35	0.54±0.09
1293	Thymol	59.89±2.97	55.65±1.73	53.70±4.98	63.63±12.31	62.15±3.26
1303	Carvacrol	3.36±0.24	2.86±0.43	6.69±5.16	3.49±0.86	3.54±0.10
1358	β-Bourbonene	t	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
1417	β-Caryophyllene	2.39±0.56	2.20±0.70	2.30±0.74	2.89±1.24	1.99±0.65
1439	Aromadendrene	0.20±0.09	0.21±0.04	0.18±0.02	0.24±0.08	0.27±0.05
1454	α-Humulene	0.25±0.07	0.32±0.09	0.25±0.06	0.28±0.08	0.21±0.06
1473	Germacrene-D	0.28±0.13	0.44±0.17	0.35±0.12	0.35±0.16	0.25±0.07
1496	Bicyclogermacrene	0.08±0.07	0.10±0.09	t	0.06±0.05	0.06±0.05
1503	β-Bisabolene	t	0.00±0.00	0.00±0.00	0.11±0.13	0.00±0.00
1510	γ-Cadinene	0.27±0.03	0.14±0.12	0.22±0.09	0.30±0.08	0.18±0.04
1522	δ-Cadinene	0.35±0.12	0.32±0.11	0.31±0.10	0.39±0.14	0.27±0.07
1533	(E)-α-Bisabolene	0.37±0.04	0.37±0.10	0.27±0.05	0.33±0.06	0.31±0.08
1581	Caryophyllene oxide	0.39±0.24	0.25±0.16	0.24±0.21	0.42±0.11	0.25±0.08
1621	10-epi-γ-eudesmol	0.23±0.08	0.17±0.15	0.07±0.12	0.16±0.04	0.18±0.07
	Total (%)	98.03	95.29	98.01	97.66	98.13

RI, retention index
All data are means of three replications ± standard deviation
t, trace (<0.05%)

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