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Research Article

GROWTH AND VOLATILE OIL YIELD OF GARDEN THYME AS AFFECTED BY NITROGEN SOURCE AND LEVEL

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ABSTRACT

A greenhouse experiment was conducted to evaluate the effects of nitrogen source and level on growth characteristics, volatile oil percentage and oil yield of garden thyme in Shahin Shahr, Isfahan province, Iran. The pots were treated using 50 or 100 mg N/kg (100 or 200 kg N/ha) by two sources of nitrogen, ammonium nitrate (33% N) and urea (46% N), and compared to control (without using nitrogen). The experiment was carried out using a Completely Randomized Design (CRD) with three replications. The results indicated that the source and level of nitrogen fertilizer altered growth characteristics, oil percentage and oil yield of garden thyme significantly. The highest value of shoot dry weight (15.04 g/plant) was obtained at 100 mg N/kg of ammonium nitrate which was significantly different when compared to other treatments. The maximum of volatile oil percentage (0.80%) was obtained at 100 mg N/kg of ammonium nitrate which was not significantly different when compared to 50 mg N/kg of ammonium nitrate. The highest value of volatile oil yield (120.26 mg/plant) was achieved on 100 mg N/kg of ammonium nitrate which was significantly different when compared to other treatments.

Keywords: *Thymus Vulgaris, Urea, Ammonium Nitrate, Medicinal Plants, Essential Oils*

INTRODUCTION

Thyme (*Thymus vulgaris* L.) from family Lamiaceae (Labiatae) is perennial plant. The green part of the shoot contains the most popular herbal medicine and spice, used in all developing countries. It is used for its pharmacological activities and has a very important role in phytotherapy (Razic *et al.*, 2003).

Volatile oil percentage of thyme has been reported from 0.32% (Ozguven and Tansi, 1998) to 4.9% (Carlen *et al.*, 2010). In flowering samples of thyme in eastern Morocco (Taforalt), essential oil yield was 1.0% (Imelouane *et al.*, 2009).

This plant 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).

Thymol and carvacrol are the active substances of thyme oil (Atti-Santos *et al.*, 2004; Goodner *et al.*, 2006) and act as antimicrobial agent (Deans and Ritchie, 1987; Prabuseenivasan *et al.*, 2006), antifungal agent (Klaric *et al.*, 2007), antioxidant (Dorman *et al.*, 1995; Jukic and Milos, 2005; Kulisic *et al.*, 2005), treatment for respiratory tract diseases (Inouye *et al.*, 2001), wound healing, a stomachic carminative, diuretic and urinary disinfectant (Boskabady *et al.*, 2006).

Plants take nutrients from the soil during their growth. Nitrogen is a macro element results in the largest growth and yield response in medicinal plants (Cox, 1992; Ayub *et al.*, 2011).

Nitrogen source affected active substance (withanolide-A) production from cell suspension cultures of *Withania somnifera* (Nagella and Murthy, 2011). Omer *et al.*, (2008) indicated that nitrogen sources altered herb yield and essential oils of *Ocimum americanum*, a medicinal plant. Misra and Gupta (2006) revealed that alkaloid accumulation in *Catharanthus roseus* seedlings was affected by nitrogen sources. Increased accumulation of 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.

The subject of this study was evaluation of the effects of nitrogen sources and levels on growth and volatile oils of garden thyme.

MATERIALS AND METHODS

Plant materials and experimental conditions

This experiment 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 pots were treated using 50 or 100 mg N/kg (100 or 200 kg N/ha) by two sources of nitrogen, ammonium nitrate (33% N) and urea (46% N), and compared to control (without using nitrogen). Experiment was carried out using a completely randomized design (CRD) with three replications. Each replicate contained 5 pots. The plants were harvested at full bloom stage, 10 cm above the pot soil surface for measurement of shoot fresh weight and were dried at room temperature for determining shoot dry weight. Soil particles were separated from the roots for measurement of root fresh weight. Root dry weight was determined after drying for 4 days at 55°C.

Volatile oil isolation

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

Statistical analysis

All data from the experiment were subjected to analysis of variance (ANOVA) using SAS computer software and the means compared with Duncan's new multiple range test (DNMRT) at $P < 0.05$.

RESULTS AND DISCUSSION

The source and level of nitrogen fertilizer altered growth characteristics, oil percentage and oil yield of garden thyme significantly (Table 1). Shoot fresh weight was the maximum (45.40 g/plant) at AN_{100} which was not significantly different when compared to U_{100}. The highest value of shoot dry weight (15.04 g/plant) was achieved on AN_{100} which was significantly different when compared to other treatments. AN_{100} resulted in the highest values of root fresh and dry weights (4.62 and 0.73 g/plant, respectively). The maximum of volatile oil percentage (0.80%) was obtained at AN_{100} which was not significantly different when compared to AN_{50}. The highest value of volatile oil yield (120.26 mg/plant) was achieved on AN_{100} which was significantly different when compared to other treatments.

Table 1: Effects of nitrogen sources and levels on growth, oil percentage and oil yield of garden thyme

Nitrogen (mg N/kg)	Shoot FW (g/plant)	Shoot DW (g/plant)	Shoot height (cm)	Root FW (g/plant)	Root DW (g/plant)	Volatile oil percentage	Volatile oil yield (mg/plant)
Control	31.12c	9.72d	30.77c	1.74d	0.38c	0.52b	50.15d
U_{50}	40.45b	12.59bc	35.51b	3.26b	0.50b	0.57b	72.03c
U_{100}	43.48a	13.27b	41.86a	2.44c	0.34cd	0.46b	61.74cd
AN_{50}	38.50b	11.21c	42.73a	1.76d	0.29d	0.79a	88.20b
AN_{100}	45.40a	15.04a	33.29bc	4.62a	0.73a	0.80a	120.26a

Abbreviations: U, urea; AN, ammonium nitrate. In each column, means with the same letters are not significantly different at 5% level of Duncan's new multiple range test.

The source of nitrogen can influence growth and active substances of medicinal plants. High nitrogen levels may decrease medicinal plant growth and secondary metabolite accumulation (Hornok, 1983; Laughlin, 1983; Boyle and Craker, 1991). Our results are in agreement with previous studies reported by researchers. The studies indicated that 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). Nitrogen can influence yield and quantity and composition of volatile oils of basil plants (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.

Application of nitrogen depends upon crop, cultivar, soil characteristics and fertility status of soil (Ayub *et al.*, 2011).

Sharafzadeh (2011) reported that nutrients affected growth and volatile oils of thyme. Geographical conditions and the age of plants can affect active substances. In a study with three years old thyme plants, herb yield and oil content were affected by nitrogen and phosphorus levels (Omidbaigi and Arjmandi, 2002).

An experiment regarding the fennel, a medicinal plant, illustrated that application of different sources of nitrogen had significant effects on both nitrate reductase activity and nitrate reductase gene expression (Sharifi Rad *et al.*, 2013).

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).

Conclusion

Nitrogen at 100 mg/kg (200 kg/ha) of ammonium nitrate resulted in the best values of growth and essential oil percentage and yield, therefore can be recommended under present experimental conditions.

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