Industrial Crops & Products 121 (2018) 434–440

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Application of combined fertilizers improves biomass, essential oil yield, aroma profile, and antioxidant properties of *Thymus daenensis* Celak.

Zohreh Emami Bistgani[a], Seyed Ataollah Siadat[a], Abdolmehdi Bakhshandeh[a], Abdollah Ghasemi Pirbalouti[b], Masoud Hashemi[c], Filippo Maggi[d], Mohammad Reza Morshedloo[e,*]

[a] Ramin Agriculture and Natural Resources University of Khozestan, Ramin, Iran
[b] Department of Medicinal Plants, Research Center for Medicinal Plants & Ethnoveterinary, Shahrekord Branch, Islamic Azad University, PO Box: 166, Shahrekord, Iran
[c] Stockbridge School of Agriculture, University of Massachusetts, Amherst, MA, USA
[d] School of Pharmacy, University of Camerino, Camerino, Italy
[e] Department of Horticultural Science, Faculty of Agriculture, University of Maragheh, 55136-553 Maragheh, Iran

ARTICLE INFO

Keywords:
Thymus daenensis
Fertilizers
Essential oil yield
Aroma profile
Thymol
Antioxidant activity

ABSTRACT

Thymus daenensis Celak., also known as denaian thyme, is an endemic and endangered medicinal herb, having valuable therapeutic properties in the Iranian traditional medicine. In the present study, a two years' field experiment was conducted to study the influence of organic and inorganic fertilizers on biomass, essential oil yield, essential oil content and compositions and antioxidant activity of denaian thyme in southwest of Iran. The treatments were: no fertilizer (control), chemical fertilizer (CF; NPK at 100-150-100 kg ha^{-1}), cow manure, vermicompost (VC) and combined fertilizers (chemical fertilizer + cow manure + vermicompost). Application of combined fertilizers significantly increased the biomass and essential oil yield of denaian thyme as compared with control, in the first and second year, by 39.61% and 68.62%, respectively. Application of fertilizers, however, significantly affected the essential oil compositions of the plants in both growing years. The essential oil of *T. daenensis* was rich in the phenolic monoterpene thymol. The highest amount of thymol was obtained under application of combined fertilizers in both years. The antioxidant activity of *T. daenensis* methanolic extracts, evaluated by the DPPH assay, increased (10.41% compared with control) under application of combined fertilizers in the second year. In both years, the N content of the soil increased after treatment with combined fertilizers (around 43% higher over the control). Alike, soil fertility parameters such as P and K showed a marked improvement in all treatments over the control. Results of this study showed that the combined application of fertilizers enhanced biomass and essential oil yield and improved the soil characteristics, antioxidant properties and aroma profile.

1. Introduction

The management of plant nutrition is one of the most important strategies for agricultural development. Understanding the relationships between different nutrients, and the combination of mineral and organic fertilizers is of crucial importance to improve the crop yields and production in an environmentally sound manner (FAO, 2007). In arid and semi-arid areas of the world, such as Iran, the organic matter level of the soil is usually very low. Thus, conservation and improvement of the soil fertility is of pivotal importance for maintaining soil health and sustainability of farming (Keshavarz Afshar et al., 2014).

Nowadays, there is a challenge in Iran to increase the herb and essential oil productivity of *T. daenensis* through improvement of agricultural practices without increasing the cultivation area. Plant nutrition is one of the most important factors affecting the secondary metabolism including the terpenoid components giving the aroma profile to essential oil bearing plants (Pandey et al., 2015; Patel et al., 2015). In addition, it has been reported that nutrition plays a pivotal role in plant growth, yield and development (Keshavarz Afshar et al., 2014; Pandey and Patra, 2015). In the case of medicinal and aromatic plants (MAPs), application of fertilizers could enhance effectively the essential oil yield and content (Aziz et al., 2010; Jabbari et al., 2011). Actually, crops react significantly to the application of nitrogen (N) fertilizer (Singh et al., 2007). In recent decades, the usage of organic fertilizers in developing countries to increase the crop productivity has been intensified (Patel et al., 2015). However, the organic fertilizers

* Corresponding author.
E-mail address: morshedloo@maragheh.ac.ir (M. Reza Morshedloo).

https://doi.org/10.1016/j.indcrop.2018.05.048
Received 10 January 2018; Received in revised form 12 May 2018; Accepted 20 May 2018
0926-6690/

give rise to potential environmental and human health concerns (Keshavarz Afshar et al., 2014). Generally, organic manure has been applied to enhance crop production for centuries (Pandey and Patra, 2015). It is nowadays recognized that using vermicompost as an organic amendment in crop production is commercially possible and profitable (Singh and Wasnik, 2013).

Organic fertilizers are extensively used to improve the agricultural production (Zandvakili et al., 2017). Although the effect of different organic and chemical fertilizers in increasing plant yield has been well documented (Pandey and Patra, 2015), there is little information regarding the combination effects given by the concurrent use of chemical and organic fertilizers on plant yield and essential oil production in MAPs (Pandey et al., 2015). Recently, MAPs have attracted attention for their potential use as source of antioxidants able to neutralize toxic free radicals (Ghasemi Pirbalouti, 2009). Therefore, efforts in the search and enrichment of natural antioxidants in MAPs have been increased (Ghasemi Pirbalouti et al., 2012).

The genus *Thymus* L. (thyme), belonging to the Lamiaceae family, encompasses perennial herbs and small shrubs distributed mainly in the Mediterranean area (Cronquist, 1988). In Iran, the genus consists of 14 species which are distributed in different climatic areas. Among them, *T. daenensis* Celak subsp. *daenensis* Celak, *T. daenensis* Celak subsp. *lancifolius* (Celak.) Jalas, *T. carmanicus* Jalas, *T. persicus* (Roniger ex Reach. F.) and *T. trautvetteri* Klokov and Desj-Shost are recognized as endemisms (Rechinger, 1963-1998; Mozaffarian, 2012). *Thymus* species are well-known for their pleasant aroma and flavor as well as sources of bioactive compounds so that they are very popular as medicinal and flavoring agents (Nickavar et al., 2005; Ghasemi Pirbalouti et al., 2012; Dall'Acqua et al., 2017).

Thymus daenensis Celak., commonly known as denaian thyme, is an economically important culinary herb in Iran (Mozaffarian, 2012). Indeed, it is widely used as a flavoring agent (condiments and spices) in herbal teas, and also as a medicine (Nickavar et al., 2005; Ghasemi Pirbalouti et al., 2012). *Thymus* species are well-known for their strong antimicrobial and antioxidant activities (Ghasemi Pirbalouti et al., 2014). In the Iranian traditional medicine denaian thyme is used as carminative, digestive, antispasmodic, anti-inflammatory and expectorant agent (Nickavar et al., 2005; Ghasemi Pirbalouti, 2009). Essential oil of *T. daenensis* has different pharmacological properties, including anti-viral (Saderi and Abbasi, 2011), anti-bacterial (Ghasemi Pirbalouti et al., 2011), anti-fungal (Ghasemi Pirbalouti et al., 2012), antioxidant (Amiri, 2012), insecticidal (Gavadi Elmi et al., 2007) and immunomodulatory (Ghasemi Pirbalouti et al., 2011). The aromatic profile of this species is characterized by phenols, aromatic and non aromatic monoterpenes such as thymol and carvacrol and their biosynthetic precursors *p*-cymene and γ-terpinene, respectively. These components not only are responsible for the aroma and flavor of the herb but also significantly contribute to its biological effects (Ghasemi Pirbalouti et al., 2014; Vitali et al., 2016).

In the present study, a field experiment was conducted to determine the effects of (1) different chemical fertilizers (N, P, K); (2) organic manure (cow manure and vermicompost) and (3) integrated fertilizers (N, P, K, cow manure, vermicompost) on biomass yield, essential oil quality and antioxidant activity of *T. daenensis*.

2. Materials and methods

2.1. Plant materials and growth conditions

Thymus daenensis seeds were obtained from the Pakan Seed Co., Isfahan, Iran. A voucher specimen was collected at flowering stage, and deposited in the herbarium of Esfahan Agriculture and Natural Resources Research Center, Iran, with voucher number of 6268. The identification was performed by Mohammad Taghi Feyzi (a plant taxonomist). The seeds were germinated in a coco peat: perlite mix (70:30, w:w) contained in plastic germination trays in greenhouses at the experimental farm of Islamic Azad University of Shahrekord, Iran (N 32°20′, E 50° 51′, 2061 m a.s.l.). The climate of the experimental site is cold and semi-arid with annual average temperature of 12 °C. Eight weeks after seeding, seedlings with uniform size were transplanted into the main experimental plots containing silty clay soil in May 2014. The soil pH was 7.6 (Table 1).

Table 1
Physico-chemical properties of field soil (depth of 0–30 cm), cow manure and vermicompost.

Parameter	Field soil	Cow manure	Vermicompost
EC (dS m^{-1})	0.04	1.0	0.9
pH	7.6	8.3	8.0
Organic carbon	0.42%	36.46%	29.9%
Total N	0.02 (%)	2.03%	3.2%
Available P	51×10^{-4} (%)	0.3%	0.3%
Available K	0.02 (%)	0.5%	0.4%

2.2. Treatments details

A field experiment was conducted in two growing seasons (2014 and 2015) based on randomized block design with five treatments and three replications. The treatments were: control (without fertilizer), CF (chemical fertilizers 100-150-100 kg ha^{-1} N, P, K, respectively), CM (cow manure 20 t ha^{-1}), VC (vermicompost 10 t ha^{-1}), and CF + CM + VC (chemical fertilizers, 33.33-50-33.33 kg ha^{-1} N, P, K) + cow manure (6.6 t ha^{-1}) + vermicompost (3.33 t ha^{-1}). N, P and K were applied as urea (CO-$(NH_2)_2$), triple superphosphate (Ca $(H_2PO_4)_2$) H_2O and potassium sulphate (K_2SO_4), respectively. Cow manure, vermicompost, and chemical fertilizers were applied to the soil before transplanting. The ratio of CF and organic manures in different treatments was determined on the basis of optimum nutrient requirement of the plant, soil properties of the experimental site, and nutrient concentration of organic manures (Patra et al., 2000).

Plots were irrigated immediately after transplanting and throughout the growing seasons when needed. No pesticide was used during the experiment, and weeds were controlled manually. In both years, plants were harvested at full bloom stage and 5 cm above the soil surface. In the first and second year, the harvests were performed on 13 September and 26 June, respectively.

2.3. Soil nutrient analysis

After plant harvesting, soil samples were taken from three randomly selected sites in each plot from 0 to 30 cm of depth. The samples were homogenized, mixed and passed through a 2 mm filter for determination of soil chemical characteristics (Table 1). Total N was determined by the Kjeldahl method (Page, 1982). Available P content was determined spectrophotometrically after extraction in sodium bicarbonate as explained by Olsen et al. (1954). Potassium was extracted with ammonium acetate and analyzed by flame photometer (Black, 1965).

2.4. Essential oil extraction

The plants were harvested at 4 cm above the soil surface and then air-dried at room temperature for ten days. The dried plants were weighed and then ground into a powder using an electric grinder. Fifty g of each ground sample were subjected to hydrodistillation using a Clevenger-type apparatus for 3 h (Clevenger, 1928). Isolated essential oils were dried over anhydrous sodium sulphate, sealed in dark vials and kept at 4 °C until GC-FID and GC–MS analyses. Hydrodistillation was performed in triplicates (n = 3) and the essential oil content (%, v/w) and yield (g m^{-2}) were estimated according to the following equations: