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ORIGINAL ARTICLE

Chlorophyll *a*, Chlorophyll *b* and Carotenoids of Garden Thyme (*Thymus vulgaris* L.) as Affected by Nutrients

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ABSTRACT

Garden thyme (*Thymus vulgaris* L.) is a herbaceous 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. Environmental factors such as soil nutrients show important role in growth and active substances of medicinal plants. Pigments (chlorophyll and carotenoids) are very important group of primary metabolites that have important role in process of photosynthesis and plant protection from extensive radiation. They have huge appliance in pharmaceutical industry, cosmetology and dietetic. A greenhouse experiment was conducted to evaluate the effect of nutrients on pigment contents of thyme. The treatments were using N, P, K, NP, NK, PK, NPK and Agrihansa (a complete fertilizer, 20-20-20) and comparing them to control (without using fertilizers). The results indicated that maximum chlorophyll *a* (4.89 mg/g dw) was observed in Agrihansa treatment . The maximum chlorophyll *b* (2.05 mg/g dw) was achieved on Agrihansa treatment which was not significantly different when compared to NP and NPK. The highest carotenoids (0.92 mg/g dw) were obtained in Agrihansa treatment. Chlorophyll *a*+*b* had the highest value (6.94 mg/g dw) in Agrihansa treatment which was not significantly different when compared to NPK treatment. The maximum chlorophyll *a*+*b*/carotenoid (8.02) was achieved in NPK treatment and chlorophyll *a*/*b* (2.38) in Agrihansa treatment.

***Key words*:** medicinal plants, pigments, Lamiaceae, Agrihansa, fertilizers.

Introduction

Thyme (*Thymus vulgaris* L.) is a herbaceous perennial plant belonging to the Lamiaceae family. The plant is native to the western Mediterranean region and southern Italy [13]. There are 350 species of thyme cultivated all over the world [24]. The green part of thyme plant constitutes the most popular herbal medicine and spice, used in all developing countries. The beneficial effects of thyme are well known from ancient times and consumption of its extract is recommended all over the world [1]. It is considered as the main ingredient of many phytopreperations and commonly used as water extracts for its pharmacological activities and thus, have a very important role in phytotherapy [17]. Recently, thyme has become one of the most important medicinal plants used as a natural additive in poultry and livestock feeding studies [3,8]. Such studies have shown that thyme plant could be considered as an alternative natural growth promoter for poultry instead of antibiotics [14]. Thymol and carvacrol, which are the principal constituents of thyme oil [2] have been reported to act as antioxidant [11], antimicrobial agent [6,16], antifungal agent [10] treatment for respiratory tract diseases [9], wound healing, a stomachic carminative, diuretic and urinary disinfectant [4].

Plant pigments (chlorophyll and carotenoides) are very important group of primary metabolites. Besides their role in process of photosynthesis and plant protection from extensive radiation, they have huge appliance in pharmaceutical industry, cosmetology and dietetic. Plant pigments are also given significant role in antioxidant activity[18,19]

Plants are being used for improving the quality of diet and sustaining or regulating good health. Herbal medicines not only provides nutrients but also strengthen and supports the action of digestive system, speeding up the rate of proceeding food and improving the absorption of nutrients. Pigments found in plants play important roles in plant metabolism and visual attraction in nature. Major plant pigments include carotenoids, anthocyanins and other flavonoids, betalains and chlorophylls. They are also rich in flavonoids and other phenolic

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constituents. Their antioxidant activity makes them chemo preventive and adds various other medicinal values to them. Plant pigments can be used fields as natural food colourings but they act as antioxidants against free radical to support immune system and prevent diseases [23]. Soil nutrients, macro and microelements, show important role in growth and active substances of medicinal plants [20,21]. Sharafzadeh *et al.* [22] indicated that nutrients changed chlorophyll and carotenoid contents of lemon balm, a medicinal plant.

The subject of this study was application of various nutrients, alone and together in order to determine their effects on chlorophyll and carotenoid contents of garden thyme.

Materials and Methods

Plant material and experimental conditions:

This study was conducted on experimental greenhouse of Firoozabad Branch, Islamic Azad University, Iran (28°35' N, 52°40' E; 1327 m above sea level). Breeded seeds were sown and the plants were transplanted in pots containing 1/3 soil, 1/3 sand and 1/3 peat (v/v) at 4-6 leaves stage and kept at 27±3/17±3°C day/night temperatures. The treatments were using N, P, K, NP, NK, PK, NPK and Agrihansa (a complete fertilizer, 20-20-20) and comparing them to control (without using fertilizers). N, P_2O_5 and K_2O, with using ammonium nitrate (33% N), triple super phosphate (46% P_2O_5) and potassium sulphate (50% K_2O), were applied at a concentration of 50 mg/kg of pot soil (approximately 100 kg/ha). The amounts of ammonium nitrate, triple super phosphate, potassium sulphate and Agrihansa were calculated as 151.5, 108.7, 100 and 250 mg/kg of pot soil equal to 545.4, 391.3, 360 and 900 mg per each pot (contained 3.6 kg soil), respectively. The soil of pots were tested before applying treatments and soil texture was sandy-loam with PH=7.73, organic C=2.27%, total N=0.22%, available P=20.19 mg/kg, available K=176.7 mg/kg and EC=1.94 dS/m. Experiment was carried out using a randomized complete block design (RCBD) with four replications. Each replicate contained 5 pots. After six months plants were cut to 10 cm above the pot soil surface and were dried at room temperature. Drug leaves was separated from the stem by hand.

Pigment determination:

In order to determine pigments content, 250 mg of drug leaves of each replication were ground and pigments were extracted in 80% acetone. Acetone was added to a volume of 50 ml. The volume was centrifuged for 10 minutes and then absorbance of samples was measured at 470, 646 and 663 nm using a Perkin Elmer UV-vis spectrophotometer. Chlorophyll *a*, chlorophyll *b* and carotenoids were calculated using the method of Lichtenthaler and Wellburn [12] and expressed per dry weight.

Statistical analysis:

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

Results and Discussion

The results indicated that maximum chlorophyll *a* (4.89 mg/g dw) was observed in Agrihansa treatment which was significantly different when compared to other treatments. The maximum chlorophyll *b* (2.05 mg/g dw) was achieved on Agrihansa treatment which was not significantly different when compared to NP and NPK. The highest carotenoids (0.92 mg/g dw) were obtained in Agrihansa treatment and the lowest carotenoids (0.56 mg/g dw) were observed in control. Chlorophyll *a+b* had the highest value (6.94 mg/g dw) in Agrihansa treatment which was not significantly different when compared to NPK treatment. The maximum chlorophyll *a+b*/carotenoid (8.02) was achieved in NPK treatment and chlorophyll *a*/*b* (2.38) in Agrihansa treatment (Table 1).

Redzic *et al.* [15,16] reported that the ratio between chlorophyll *a* and chlorophyll *b* in some medicinal plants of Lamiaceae family is rarely 3:1, as stated in classical literature but rather close to 3:2 and more, which makes these species even more medical and gives them higher potential anti-oxidant capacity [5]. Another study, with the same treatments, indicated that NK treatment resulted in the highest values of chlorophyll and carotenoids in *Melissa officinalis* L. plants [22] but the result of present study was different.

Agrihansa (complete fertilizer) treatment produced the highest level of pigments. It is already established that leaf chlorophyll content increases with N supply. Phosphorus is a part of phospholipids, nucleic acids and nucleoproteins. Potassium is an ion that activates various enzymes and play important role in protein synthesis. Anions transport, assimilate translocation, cell respiration and stomatal movements are influenced by Potassium. On the other hand, magnesium is the central molecule in chlorophyll and is an important cofactor for the production of ATP. As S is an essential constituent of certain amino acids and proteins, S deficiency results in the inhibition of protein and chlorophyll synthesis. Fe deficiency reduces chlorophyll Production. Copper is needed for chlorophyll production, respiration, and protein synthesis [25]. An investigation with broccoli showed that chlorophyll content was higher when inorganic fertilizer adding with organic manure compared with using organic manure alone [15]. Another study with

Celosia argentea indicated that nitrogen fertilizer increased total chlorophyll and carotenoids [7].

Different species can respond to nutrients differently. Nutrients can alter pigments accumulation. On the other hand, several internal and external factors such as genotype, physical and chemical characteristics of the soil, light and temperature may interact with nutrients. Under the conditions of this experiment, Agrihansa resulted in the highest values of the pigments and followed by NPK treatment. Imbalance of nutrients in some of treatments could not increase pigments when compared to Agrihansa and NPK treatments.

Table 1: Effect of nutrient treatments on pigments of thyme drug leaves.

treatment	Chl *a* (mg/g dw)	Chl *b* (mg/g dw)	Car (mg/g dw)	Chl *a*+*b* (mg/g dw)	Chl *a*+*b*/Car	Chl *a*/Chl *b*
Control	2.89f	1.38d	0.56f	4.27f	7.69a	2.10cd
N	4.18cd	1.83b	0.79bc	6.02c	7.59a	2.28ab
P	3.53e	1.61c	0.71de	5.14e	7.24a	2.19bcd
K	3.67e	1.70bc	0.73cde	5.36de	7.37a	2.16bcd
NP	4.45bc	2.01a	0.83b	6.47b	7.81a	2.21bc
NK	3.96d	1.73bc	0.76bcd	5.69cd	7.55a	2.29ab
PK	3.55e	1.72bc	0.68e	5.27e	7.75a	2.07d
NPK	4.53b	2.03a	0.82b	6.57ab	8.02a	2.23bc
Agrihansa	4.89a	2.05a	0.92a	6.94a	7.54a	2.38a

In each column, means with the same letters are not significantly different at 5% level of Duncan's new multiple range test (DNMRT).

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