Nitrogen and Iron Fertilization Methods Affecting Essential Oil and Chemical Composition of Thyme (*Thymus Vulgaris* L.) Medical Plant

Reza Jabbari, Majid Amini Dehaghi, Ali M. Modares Sanavi, Kayvan Agahi

*ORIGINAL ARTICLE*

**ABSTRACT**

Nutrition plays a key role on the amount of essential oil compounds among medical plants. The main objectives of this research is to evaluate the different application methods of Iron and Nitrogen nutrition elements on essential oil and chemical composition of thyme (*Thymus vulgaris* L.). The experiment was carried out at the research fields of medical plants, of shahed University, Tehran, Iran, in 2008. The effect of Nitrogen and Iron elements on thyme seedlings was assayed. Two randomized complete block designs with four replications. Treatments included soil and foliar application of these elements. Results showed that Nitrogen foliar application increased the vegetative yield, amount and percentage of essential oil and chemical compositions of thyme. On the other hand, application of Iron had a suppressing effect on the studied traits.

**Key words:** Thyme (*Thymus vulgaris* L.), thymol, carvacrol, p-cymene, nitrogen, iron.

**Introduc**

Essential oils are extractable from different parts of medical plants and highly usable as food flavours [11]. Such oils, which are also called Volatile oils, have some interesting usage as medical materials with antibacterial, antifungal, antiviral, insecticidal and antioxidant effects[26]. These medical oils can be used for cancer treatment[42], food preservation [15], aromatherapy[12] and production of fragrance [44]. Thyme is a medical plant with producing high quality essential oil with aromatic properties, used by different industries including pharmaceutical, food and cosmetic[23,2].

This medicinal plant has been indicated to have the following pharmacological effects: 1) antispasmodic, 2) expectorant, 3) antiseptic, 4) antimicrobial, 5) and antioxidant[31,27]. Thyme may have the followin chemical compounds: thymol, thymol/carvacrol and thymol/carvacrol/ linalool[9,36]. Research carried out in Sicily[10,39], Sardinia [19,5] and Albania[13,32] showed that the local populations of thyme contain carvacrol and small quantities of thymol, often lower than 1%.

Nitrogen (N) fertilizer is important for optimum yield production affecting different plant growth parameters including leaf area development[8,19]. Alkire and Simon[3] and Piccaglia *et al.*[37] indicated that N enhances the oil yield quantity of peppermint through affecting different growth

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parameters including tillers per plant, the total plant dry weight and the leaf Area Index (LAI).

Although iron is among the most important and abundant micronutrients for crop production worldwide, its limited availability usually adversely affects plant growth and yield production[46]. Plants subjected to Fe deficiency/excess may use different strategies to respond in [1,38]. For example, under Fe deficiency, some plants can absorb higher rates of Fe. The related mechanisms affecting Fe availability usually become active under aerobic conditions where Fe (III) is present[30].

This study provides some more appropriate Fe and N management, which in turn can be used for cost-effective applications of fertilizers thereby leading to higher yield.

Materials and methods

This study was performed in two different Research Fields of Medical Plants, University of Shahed, Tehran, Iran, in 2008 (48°53' N–31°36' W and 1050 m above sea level). Soil texture in the surface 30 cm of the cultivation area contained 42% sand, 28% and 30% clay with pH 7.2 and total N at 0.196%. Semiarid climatic conditions are characterized by an annual maximum temperature of 40 °C and minimum temperature of -8.5 °C and average rainfall of 259.5 mm/year. A total of 30 plants in each plot were cultivated with a planting distance of 0.2 ×0.5 m. During the full bloom stage five plants per plot were harvested. The effect of N and Fe on the quantitative and qualitative growth of thyme seedlings was assayed by conducting two different experiments on the basis of randomized complete block designs with four replications. N treatment was applied at control and 50 kg.ha-1 for both soil and foliar applications and Fe treatment was applied at control and 5 g.m2 -1 for both soil and foliar applications.

Aerial parts of *Thymus vulgaris* and were purchased from the Research Center of Medicinal Plants, Tehran, Iran.

Essential oil extraction

The aerial dried parts of thyme were subjected to steam distillation in a Clevenger-type apparatus for 3 h. The essential oils obtained were separated from water and dried over anhydrous Na2SO4 then stored at 4 °C until use.

Gas chromatography

Samples of 0.1µL were subjected in quadruplicate to be analysed by capillary gas chromatography. A Unicum series 4880 gas chromatograph (GC), equipped with a flame ionization detector (FID) and a 2 m×0.3 mm pack OV, was used for this study. The FID and the injector, maintained in 250 °C N, was used as carrier gas. The column was maintained at 60 °C for 2 min, increased to 195 °C at a rate of 8 °C/min and finally raised to 195 °C. N was used as the carrier gas at a constant flow rate of 1.0 ml.min⁻¹.

Standards

Standard thymol pure sample was supplied by Merk and the standard carvacrol and P-cymene pure samples were supplied by Aldrich. The graph peak for thymol, carvacrol and P-cymene were observed after 10’47” and 11’ and 4’7”, respectively. The results were calculated on the basis of composition percent in 5% of essential oil (Fig1).

Fig.1. Structures of the monoterpene HC precursors (*p*-cymene) and their biosynthetic products: monoterpene phenols (thymol and carvacrol), quality marker constituents of thyme oil.

Statistical analysis

Data were subjected to analysis of variance using SAS 9.2 program. Comparisons of means were performed using Duncan’s multi-range test.

Results and discussion

Experiment 1

Total dry matter (TDM)

The results showed that N fertilizer application method significantly affected TDM (Table 1). The most effective method of N fertilizer application was foliar application producing significantly higher TDM (2488.4 Kg.ha⁻¹) than the control treatment (1130.2 Kg.ha⁻¹) (Table 2). The observed significant differences in TDM among different methods of fertilizer application, especially between low and high rates, were probably due to the differences in herb size. The control treatment (no N application) decreased the vegetative development during the phonological stage and delayed the natal stage delay as well as the propagation and substance leaf area. Accordingly, the sun light efficiency decreased [43]. Jeliazkova *et al.* [24] reported higher TDM production with increasing the accessible rate of NPK fertilizer application. Foliar application of N increased TDM production due to enhanced N accessibility.

Essential oil yield

Method of N fertilizer application significantly affected the essential oil yield (Table 1). Higher essential oil yield (38.21 L.ha⁻¹) was obtained under foliar application of 50 kg N.ha⁻¹ than the control (20.39 L.ha⁻¹) (Table 2). Also N soil application
increased essential oil yield but lower than foliar application. The research performed by Hornok [22] and Drazic and Pavlovic [14] indicated relation between photosynthesis area and essential oil, which is in agreement with our results (Table 3).

**Essential Oil Composition**

The different methods of N application had significant effects on the composition of thyme essential oil (Table 1). The compositions of thyme essential oil, hydrodistilled from the herb harvested from different methods of N fertilizers, were analyzed by GC. The results obtained indicated that N foliar application increased, thymol percentage (2.56 %) and thymol (19.56 Kg.ha$^{-1}$), carvacrol (0.53 Kg.ha$^{-1}$), and p-cymene (1.18 Kg.ha$^{-1}$) yield (Table 2). Control leads to more p-cymene, carvacrol percentage compared to N application. P-cymene yield was increased about 1.19 and 1.18 Kg.ha$^{-1}$ for control and foliar application treatments, respectively (Table 2).

Thymol was the most abundant compound in all analyzed oils, followed by p-cymene and carvacrol. Barnauskiene et al. [6] reported that thymol, carvacrol and p-cymene were significantly affected by different N fertilizer levels application; however the related percentages of these and other compounds in thyme herb subjected to different fertilization doses were not significantly affected. Also these researchers reported that the amount of thymol and p-cymene increased with increasing N levels. Accessibility and inaccessibility to nutrients with diverse composition, affects the significance level, which is in agreement with our results [18].

Also Khan et al. [25] evaluated the effects of N foliar and soil application on fennel (Foeniculum vulgare Mill.) and indicated that the scale composition was significantly affected by the application method. Sharifi and Abbaszadeh [40] found that N fertilizer significantly increased the essential oil content of Foeniculum vulgare Mill.

**Conclusions 1**

Chemical fertilizers are usually applied at higher rates relative to the optimal rates for plant growth and yield production. The unutilized fertilizers cause soil pollution. Also Herms and Mattson [21] mentioned the theory of growth differentiation balance (GDB) framework, indicating that there is a physiological balance between plant growth and production of secondary metabolite. N is an essential macronutrient, supplied by soil and is needed in relatively large amounts by plants for adequate growth as well as for the formation of amino acid, enzyme, and protein [29]. When environmental conditions are good and N levels are adequate, according to the GDB theory plant will have a favorable growth, and proteins are produced due to the process of photosynthesis as the main source for protein production. However, when environmental conditions are poor and the availability of an essential nutrient such as N is limited, according to the GDB framework although plant growth decreases due to less allocation of photosynthesis, the production of secondary metabolites, which are usually utilized by plants for processes such as storage and defense subsequently increase [21]. Using the GDB framework, it is likely to more specifically use the carbon/nutrient balance (CNB) hypothesis [7], indicating how nutrient fertilization may affect photosynthesis allocation to different plant parts. The CNB theory states that, under limited nutrient conditions, plants produce higher rate of carbon-based compounds, particularly secondary metabolites. Accordingly the CNB hypothesis indicates that at low N fertilization levels the concentrations of carbonaceous metabolites such as polyphenolic compounds increase. However, other researchers have mentioned that there may be conditions, which the production of secondary metabolites may not be affected by nutrient availability [34].

**Experiment 2**

**Total dry matter (TDM)**

According to the results the method of Fe fertilizer application had significant effects on TDM (Table 3). The foliar and soil application of Fe fertilizer significantly decreased TDM (1624.7 Kg.ha$^{-1}$, 1711.5 Kg.ha$^{-1}$) relatively to the control (2290 Kg.ha$^{-1}$) (Table 2). It has yet to be indicated that why such effects are observed, as there is little data about the role of Fe in the production of secondary metabolites [45].

**Essential oil yield**

Method of Fe fertilizer application significantly affected the production of essential oil (Table 1). Higher essential oil yield (29.08 L.ha$^{-1}$) was obtained under foliar application of 5g N m$^{-2}$ than the soil and control application (19.5, 18.4 L.ha$^{-1}$) (Table 2). Rajab Baygi et al. (2007) found that Fe application significantly decreased the production of essential oil (Origanum vulgare L.) In comparison with the control treatment.

**Essential Oil Composition**

The different methods of Fe application significantly affected the essential oil composition of thyme except carvacrol yield (table 1). The results indicated that Fe foliar application increased thymol oil percentage (0.709 %) and p-cymene yield (2.61 Kg.ha$^{-1}$) relative to the control treatment (Table 2) as it inversely affected thymol yield and (Table 2). Rajab Baygi et al reported after treating plants with
Table 1: Analysis of variance for the effects of different N fertilization methods on essential oil and chemical composition of thyme (*Thymus vulgaris* L.)

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oil yield</td>
<td>3</td>
<td>1108.34</td>
</tr>
<tr>
<td>Thymol</td>
<td>2</td>
<td>1938422.8**</td>
</tr>
<tr>
<td>P-cymene</td>
<td>6</td>
<td>3669.6</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>6</td>
<td>3669.6</td>
</tr>
</tbody>
</table>

n.s.: non-significant. *P > 0.05. **P > 0.01.

Table 2: Mean comparison related to the effects of different N fertilization methods on essential oil and chemical composition of thyme (*Thymus vulgaris* L.) using Duncan's multirange test.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDM Oil yield (L/ha)</td>
<td>Thymol (%)</td>
</tr>
<tr>
<td>Control</td>
<td>1130.2c</td>
</tr>
<tr>
<td>N Soil application</td>
<td>1544.1b</td>
</tr>
<tr>
<td>N Foliar application</td>
<td>2488.4a</td>
</tr>
</tbody>
</table>

a Means in each column followed by the same letter are not significantly different (P < 0.05).

Table 3: Analysis of variance for the effects of different Fe fertilization methods on essential oil and chemical composition of thyme (*Thymus vulgaris* L.)

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oil yield</td>
<td>3</td>
<td>764.97</td>
</tr>
<tr>
<td>Thymol</td>
<td>2</td>
<td>523163.5**</td>
</tr>
<tr>
<td>P-cymene</td>
<td>6</td>
<td>6788.47</td>
</tr>
<tr>
<td>Carvacrol</td>
<td>6</td>
<td>6788.47</td>
</tr>
</tbody>
</table>

n.s.: non-significant. *P > 0.05. **P > 0.01.

Table 4: Mean comparison related to the effect of different Fe fertilization methods on essential oil and chemical composition of thyme (*Thymus vulgaris* L.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDM Oil yield (%)</td>
<td>Thymol (%)</td>
</tr>
<tr>
<td>Control</td>
<td>2290a</td>
</tr>
<tr>
<td>Fe Soil application</td>
<td>1711.5b</td>
</tr>
<tr>
<td>Fe Foliar application</td>
<td>1624.7b</td>
</tr>
</tbody>
</table>

a Means in each column followed by the same letter are not significantly different (P < 0.05).

Fe the relative percentage of methyl chavicol decreased.

**Conclusion 2**

According to the results it appears that Fe stress (deficiency and excess) significantly reduced thyme TDM (total dry matter), oil and thymol yield. Applications of Fe at low and excess rate relative to the recommended rates are considered inefficient because such stress is a growth inhibitor and reduce plant chlorophyll content[20] and hence the process of photosynthesis[35]. Chlorophyll decrease, can also significantly affect C assimilation by decreasing photosynthesis, due to Fe stress [28].

**Reference**