NITROGEN COMPOSITION DURING VEGETATIVE AND GENERATIVE GROWTH STAGES OF TWO ORIGANUM TAXA FROM TURKEY

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SUMMARY

\emph{Origanum onites} and \emph{Origanum vulgare} ssp. \textit{hirtum} were collected from Balikesir, on the north west of Turkey. The nitrogen contents in organs such as root, stem, leaf and flower of \emph{Origanum onites} and \emph{Origanum vulgare} ssp. \textit{hirtum} taxa in vegetative and generative growth stages were investigated. Nitrogen analyses of soil and plant samples were carried out according to the Kjeldahl method. It was found that the nitrogen content among organs of \emph{Origanum} taxa changed in vegetative and generative stages, and that the soil and plant samples possessed a higher nitrogen content in vegetative stage than in generative stage. The highest nitrogen content had the flower, followed by leaf, stem and root, respectively. Furthermore, it was observed that the nitrogen contents of these \emph{Origanum} taxa were not very different, and that the nitrogen content in root and stem was deficient and that in leaf and flower was enough for plant nutrition. In addition, there is an important relationship between the nitrogen amounts during the vegetative and generative growth stages of \emph{O. onites} and \emph{O. vulgare} ssp. \textit{hirtum} taxa. Moreover, there was an important correlation between the nitrogen amount in soil and plant.

KEYWORDS:
Soil, plant, nitrogen, \emph{Origanum}, vegetative and generative stages.

INTRODUCTION

In Europe and generally all over the world, the most commonly found oregano species belong to the botanical genus \emph{Origanum}. Forty-nine taxa divided into 10 sections belong to this genus, most of them having a very local distribution around the Mediterranean [1]. \emph{Origanum vulgare} ssp. \textit{hirtum} is a common polymorphic taxon possessing by far the largest distribution of all \emph{Origanum} species [1,2]. Its principal constituents are thymol, carvacrol, accompanied by \textit{p}-cymene and \textit{\gamma}-terpinene [1]. Due to its high content of thymol and carvacrol, the essential oil of oregano is a commonly used product in pharmacology and food industry [3]. Oregano plays a primary role among culinary herbs in world trade. The subspecies \textit{hirtum} has been more thoroughly investigated with respect to essential oil composition, since it includes high-quality, essential oil-rich, commercial taxa. At present, most of the commercial oregano from the Mediterranean area is obtained from its wild populations in Turkey and Greece, and crops in Israel. Also the wild oregano is widely utilized in southern Italy, where local ethnobotanical knowledge has led to the commercial exploitation of oregano as a food flavouring [4].

Nitrogen is necessary for the healthy development of human beings, animals and plants. The nitrogen concentrations in plants and soils may differ from part to part and from plant to plant, and even different organs of plant [5]. There are close positive relationships between nitrogen status and plant growth manifested by the close relationship between photosynthesis and nitrogen at the single leaf level, as well as for relative growth rate of the whole-plant [6].

Large areas of grassland are managed with low external inputs. Grassland species consequently are faced with nitrogen limitations and deficiencies [7]. It is necessary to assess changes in nitrogen dynamics by including all plant organs such as root, stem, leaf and flower. Høgh-Jensen et al. [7] have investigated the re-growth and nutrient composition of different plant organs in grass-clover canopies affected by phosphorus and potassium availability and found that petioles were the dominant organ with respect to dry matter, nitrogen, phosphorus and potassium. Serraj and Sinclair [8] have examined the response of N\textsubscript{2} fixation to drought stress in common bean, and tested the hypothesis whether drought sensitivity N\textsubscript{2} fixation in common bean is linked to ureide levels in the plants or not. For common bean, they found that the lower sensitivity of N\textsubscript{2} fixation to drought compared to leaf gas exchange could be related to...
low ureide concentrations in petioles and xylem sap. Glimskär [9] studied the root system topology of five plant species grown at steady-state nutrition. Also, Glimskär and Ericsson [10] have determined the relative nitrogen limitation at steady-state nutrition as a determinant of plasticity in five grassland plant species and found that the relative growth rate of the species was strongly related to plant nitrogen concentration. Moreover, it is concluded that root: shoot partitioning can be treated as a direct function of the relative resource limitation of the plant. Vouillot and Devienne-Barret [11] aimed to determine the effect of nitrogen deficiency on pre-anthesis movements of nitrogen among individual organs in winter wheat plants; this gave the results for the quantification of pre-anthesis nitrogen accumulation, and remobilization of individual organs (leaves, stem, roots) of plants, grown under field conditions, and subjected to nitrogen deficiency during stem elongation.

Alt et al. [12] have investigated the nitrogen content and the distribution in cauliflower (Brassica oleracea L. botrytis). Origanum species have been more thoroughly investigated with respect to their essential oil composition, since this family includes high-quality, essential oil-rich, and commercial species [13]. The PPO activity and protein contents of Origanum species were also investigated [14, 15]. We have found no article related to the nitrogen contents of Origanum species, even though it is necessary to characterize the nitrogen contents of the different Origanum organs, when they are used as culinary herb for the preparation of teas.

The most important nutrient for plant growth is nitrogen. The objective of this paper is to investigate the distribution of nitrogen contents of organs of Origanum onites and Origanum vulgare ssp. hirtum collected from different localities in vegetative and generative stages around Balikesir in Turkey, and to provide a data bank on nitrogen contents of field Origanum taxa. Furthermore, these Origanum taxa can also contribute to the culture of these types due to their economical importance and potential usage at different areas.

MATERIAL AND METHODS

The Origanum plants were collected around Balikesir in 2001. The localities of Origanum plants collected in this study were determined in 2000, and are given in Figure 1. A 10 cm core of soil sample, close to the Origanum growth, was collected by a stainless-steel sampler in vegetative and generative stages. Soil samples were dried at 70 °C for 24 h according to Rashed and Awadallah [5].

![FIGURE 1 - The map of study area; (♦) Origanum onites and (■) Origanum vulgare ssp. hirtum.](image_url)
The experimental plant samples were collected from the study areas in vegetative (April) and generative (June) growth periods in 2001. The plants were cleaned to remove visible soil and then washed several times with tap water and bidistilled water. The *Origanum* material was subsequently separated to root, stem and leaf in vegetative stage and to root, stem, leaf and flower in generative stage. Then, *Origanum* organs were washed again with bidistilled water, allowed for draining, and then oven-dried at 70 °C for 24 hours. They were then ground in a stainless-steel blender.

*Origanum* and soil samples were grounded and powdered with the aid of a mechanical agate mortar to 100 mesh. The powdered samples of *Origanum* plant and soil samples were stored in clean polyethylene bottles until analysis [5].

Nitrogen analyses of soil and plant samples were carried out according to the Micro-Kjeldahl method. 0.5 g of soil and plant samples was digested according to the wet ashing method and then, total nitrogen amount of samples was determined with titrimetric method [16].

The relationship among groups (root, stem, leaf, flower parts of plants and soil) was compared with one-way analysis of variance. Means were separated using the Turkey’s HSD post hoc test. Vegetative and generative stages were also compared after calculating the Pearson correlation coefficient (SPSS, 1999 – [17]). An arcsine square-root transformation was performed to obtain the percentage values of nitrogen before analysis. The results were considered as statistically significant (P<0.05). Experiments were repeated for five times. Localities were evaluated as repetition groups.

**RESULTS AND DISCUSSION**

Mean and standard error values of nitrogen in soil in vegetative and generative stages are given in Table 1. Nitrogen content for soil samples of each *Origanum* taxa varies among the five sites and between vegetative and generative stages. It is found that the nitrogen contents for soils of *Origanum onites* and *Origanum vulgare* ssp. *hirtum* taxa in vegetative stage have varied in the range of 0.23±0.02 % and 0.09±0.00 %, and those in generative stage have varied in the range of 0.14±0.02 % and 0.05±0.01 %, respectively. It has been reported that the amount of nitrogen in soil varies between 0.1 and 0.3 % in the upper 5 cm [18]. According to these results, it can be concluded that the nitrogen content of soils of *Origanum* taxa collected from different sites is enough for plant nutrition. Furthermore it is found that there is a decrease in the nitrogen contents in generative growth stage in soil and plant samples (Table 1). This is in good agreement with literature [19]. Decreasing of nitrogen contents of soil samples from vegetative stage during generative stage may be due to growing of plant on soil. Because nitrogen is taken up by plant roots from soil and combined into organic substances in the plant such as enzymes, proteins and chlorophyll. However, another reason for the decrease of nitrogen content in soil may also be denitrification, where bacteria change nitrate in the soil and release it into atmosphere as nitrogen gas. The nitrogen amount of soils, on which *O. onites* and *Origanum vulgare* ssp. *hirtum* were grown, was higher in the vegetative stage than that in the generative stage. It was observed that growth stage and nitrogen contents in soil were significantly different for *O. onites* (Table 1).

The accumulation of nitrogen in plants generally depends on its availability in soil, which determines the growth rate of the organs, its nitrogen composition, and consequently, its demand for nitrogen. The extent of nitrogen accumulation is determined by the relationships between the capacities of the plants to absorb nitrogen, to remobilize protein and to incorporate nitrogen [11].

As seen in Table 1, it is found that the nitrogen contents in different organs of both *Origanum* taxa are different from each other. The results show that the organ having the highest nitrogen for both *Origanum* taxa collected from different sites is leaf, followed by stem and root in vegetative stage, and flower followed by leaf, stem, and root in generative stage, respectively.

The percent of nitrogen of the plants was averaged from 1.2 to 3.6 % (dry weight) at different sites [20]. The major function of roots is to mediate the transfer of nutrients from soil into plants. The transfer of nutrients from soil into plants is the result of interactions between uptake by roots and transport from soil to the root surface. Transport of

**TABLE 1 - Nitrogen amounts in organs and growth soils of *Origanum onites* and *Origanum vulgare* ssp. *hirtum* (M: Means, SE: Standard errors).**

| Organs | *Origanum onites* | | | *Origanum vulgare* ssp. *hirtum* | | |
|--------|-------------------| | |-------------------| | |
|        | Vegetative | Generative | | Vegetative | Generative | |
|        | M | SE | M | SE | M | SE | M | SE |
| Root   | 0.52±0.03 a | 0.45±0.05 a | | 0.57±0.02 a | 0.50±0.04 a | |
| Stem   | 0.71±0.03 a | 0.62±0.05 a | | 0.67±0.04 a | 0.59±0.03 a | |
| Leaf   | 2.09±0.18 b | 1.24±0.09 b | | 1.82±0.09 b | 1.41±0.07 b | |
| Flower | 1.98±0.06 c | | | 1.92±0.09 c | | |
| Soil   | 0.23±0.02 c | 0.14±0.02 d | | 0.09±0.00 c | 0.05±0.01 d | |
nitrogen from soil to the root is carried out by mass flow and diffusion [21-23]. The distribution of nitrogen content in roots of *Origanum onites* and *Origanum vulgare* ssp. *hirtum* taxa collected from different sites has changed in the range of 0.52±0.03 % and 0.57±0.02 % in vegetative stage, and 0.45±0.05 % and 0.50±0.04 % in generative stage, respectively. It can be said that the nitrogen content in roots of both *Origanum* taxa is quite low. The reason of this may be that nitrogen is continuously taken up by plant roots and combined into organic substances in the plant, such as enzymes, proteins and chlorophyll.

It has also been observed that there are some differences in the amount of nitrogen for the *Origanum onites* and *Origanum vulgare* ssp. *hirtum* taxa in vegetative and generative stages depending on soil (Table 1). There is an important difference between the amounts of nitrogen among groups in vegetative (F=136.225; dF=4.20; P<0.001) and generative (F=151.841; dF=4.20; P<0.001) stages for *O. onites*. Similar to *O. onites*, it can also be said that there is an important difference between the amounts of nitrogen among groups in vegetative (F=327.043; dF=3.16; P<0.001) and generative (F=249.142; dF=4.20; P<0.001) stages for *O. vulgare* ssp. *hirtum*. The percent nitrogen ratio of root, stem and leaf has continuously increased in both stages. Furthermore, when it is not statistically important the increasing in stem according to root (P>0.05), it is important the increasing in leaf according to stem (P<0.05). There is an important correlation between the nitrogen concentrations of soils and plants.

The percent nitrogen amounts in root, stem and leaf of plant samples have shown differences between vegetative and generative growth stages. The percent nitrogen amounts in root, stem and leaf have shown a degree of increase from root to leaf at both stages (Table 1). It has been seen that p value is smaller than 0.05 which was calculated to compare the nitrogen contents of the roots of *O. onites* and *O. vulgare* ssp. *hirtum* in vegetative and generative stages. There is an important correlation related to the nitrogen concentration of roots between growth stages. The nitrogen element present in stems did not significantly change between vegetative and generative stages for both species (Table 1). Negative correlation coefficients were obtained between nitrogen amounts of leaves and growth stages (Table 2). Negative correlation was significant for *O. vulgare* ssp. *hirtum* of nitrogen amounts of leaves, whereas negative correlation was not significant for *O. onites* of nitrogen amounts of leaves. This result suggests that nitrogen in the soil may influence plant nutrient levels in plants. However, there were species-specific differences in this respect. Anderson and Eickmeier [24] stated that plant macronutrients were often associated with soil nutrient availability. The strong positive correlations were found between the nitrogen contents of *Origanum onites* and *Origanum vulgare* ssp. *hirtum* and stages (r=0.940, P=0.000 and r=0.972 P=0.000). Similar results were reported for some *Quercus* species [25]. It was determined that the nitrogen amounts in plant organs decreased during growth. This can be a result of the movement of nitrogen from root to the upper parts of plant and the increasing of carbohydrate/protein ratio in plant.

Stems provide the transport of nitrogen taken up from the soil by root to the upper organs, such as leaf and flower, of plants. The nitrogen content of *Origanum onites* and *Origanum vulgare* ssp. *hirtum* taxa collected from different sites has changed in stems in the range of 0.71±0.03 % and 0.67±0.04 % for vegetative stage, and in the range of 0.62±0.05 % and 0.59±0.03 % for generative stage, respectively; in leaves in the range of 2.09±0.18 % and 1.82±0.09 % for vegetative stage and, 1.24±0.09 % and 1.41±0.07 % for generative stage; and in flowers in the range of 1.98±0.06 % and 1.92±0.09 % for generative stage, respectively. These results show that the nitrogen content in stems of both *Origanum* taxa is low. Nitrogen in plants is combined into organic substances such as enzymes, proteins and chlorophyll. Leaves represent the nutrient store for the plants [5]. Because they continued to expand, particularly by using remobilized nitrogen from other older organs from vegetative stage during the generative stage. It can be concluded from the results that concentration of nitrogen present in the leaf and flower of *Origanum* taxa is enough, but flowers exhibit the higher nitrogen concentration than in the other organs of *Origanum* taxa. In general macroelements especially nitrogen concentration in leaves were strongly correlated with photosynthetic capacity [26]. Rashad and Avaldallah found that the nitrogen content was higher in herbs parts than woody parts. This is resulted from the mobilization of nitrogen in plants [5]. The decrease of nitrogen concentration for the all plant organs during growing was observed. This may be the results of (i) remobilization of nitrogen from the stems and lower leaves and transfer to the upper leaves of the plant and (ii) increasing carbohydrate amount according to protein content in plant. It is also observed that nitrogen content of both *Origanum* taxa is not quite different. Similar results were found by Lemaire and Salette [27].

### Table 2 - Correlation of plant parts and soil in nitrogen concentrations between vegetative and generative growth phases.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Correlation Coefficient</th>
<th>Significance</th>
<th>Correlation Coefficient</th>
<th>Significance</th>
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</thead>
<tbody>
<tr>
<td><em>Origanum onites</em></td>
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<tr>
<td>Root</td>
<td>0.932</td>
<td>0.021</td>
<td>0.911</td>
<td>0.032</td>
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<td>Stem</td>
<td>0.870</td>
<td>0.055</td>
<td>0.926</td>
<td>0.967</td>
</tr>
<tr>
<td>Leaf</td>
<td>-0.028</td>
<td>0.964</td>
<td>-0.909</td>
<td>0.033</td>
</tr>
<tr>
<td>Soil</td>
<td>0.884</td>
<td>0.047</td>
<td>0.835</td>
<td>0.078</td>
</tr>
<tr>
<td><em>Origanum vulgare</em> ssp. <em>hirtum</em></td>
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<tr>
<td>Root</td>
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<td>Soil</td>
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</table>
CONCLUSIONS

In this study the following results are obtained:

- The distribution of nitrogen content among different organs of *Origanum* taxa has changed during vegetative and generative growth period.
- The nitrogen contents in vegetative stage of *Origanum* taxa are higher than those in generative stage.
- The content of nitrogen of the *Origanum* taxa has concentrated in the younger organs than in the older organs.
- The organ including the highest nitrogen for both *Origanum* taxa in growth stages is flower, followed by leaf, stem, and root.
- The content of nitrogen of both *Origanum* taxa is not very different.
- The content of nitrogen in root and stem is deficien, and leaf and flower is enough.
- The concentration of nitrogen studied in the soil of *Origanum* taxa decreases with growth due to absorption of nitrogen by *Origanum* roots and accumulates in the different parts of the plants.

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