Effect of Nutrient Solution’s Iron Concentration on Growth and Essential Oil Content of Oregano Plants Grown in Solution Culture

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Abstract

An experiment was done with oregano [*Origanum vulgare* spp. *hirtum* (Link) Ietswaart] grown in NFT under 3 different target levels (2.5, 5.0 and 11.0 mg/l) of Fe-EDTA in the nutrient solution to study the effect of iron on growth and essential oil content of the plants. Iron uptake by plants was associated with the Fe concentration in the nutrient solution and the uptake rate during the growing season was maximal in the seed formation stage. Iron content in leaves and roots increased with increasing Fe concentration in the nutrient solution. Top (younger) leaves had less iron content than the older ones. Roots presented much higher accumulation of iron than leaves. Increasing iron concentration to 11.0 mg/l resulted in decreased plant growth parameters (fresh and dry mass of shoots, dry mass of roots), but increased the ratio of dry leaves/stem. Essential oil content of the shoots decreased in the highest (11.0 mg/l) Fe nutrient solution concentration. It can be concluded that under the conditions of the experiment the high iron concentration applied reduced both biomass and essential oil yield.

INTRODUCTION

The growing market of medicinal, aromatic plants and their derivatives has stimulated studies aimed at elucidating the factors affecting plant productivity and quality. Among other exogenous factors, fertilizers, especially macronutrients, have long been known as powerful means of increasing medicinal and aromatic plant productivity and quality (Economakis, 1993, Ichimura et al., 1995, Fournaraki, 1998, Economakis et al., 1999, Mairapetyan et al., 1999). Investigations, dealing with effect of trace elements on the performance of these plants are scarce and have primarily appeared during the last 10-20 years, mainly. Micronutrients, applied both as complex and single fertilizers have been proven effective in affecting productivity, yield of secondary metabolites, and, in some cases, quality of those metabolites (Haykazyan et al., 1994; Misra and Srivastava, 1991).

Iron is a trace element, required in higher amounts by plants than other micronutrients. The role of iron in biological redox systems (electron transfer chain in photosynthesis and respiration), enzyme activation, N₂ fixation, chloroplast development, heme proteins (cytochromes, catalase, peroxidase), iron-sulphur proteins (ferredoxin, isoenzymes of superoxide dismutase, aconitase), is well known and documented (Welch, 1995). Less is known about the effect of iron on the secondary metabolism, either indirectly, by affecting availability of photosynthates provided by primary metabolism (Srivastava et al., 1993), or directly, through some factors responsible for efficient utilization of precursors coming from primary synthesis (Srivastava et al., 1998).

The literature on the effect of iron on growth parameters and essential oil yield is contradictory. It was reported by Misra and Sharma (1991) that the optimal concentration of iron for Japanese mint (*Mentha arvensis* L.) was responsible for the fresh and dry matter yield, essential oil and menthol content. In another experiment, additional foliar application of iron gave the best results for *Cymbopogon citratus* Stapf. productivity and essential oil yield (Mairapetyan and Tadevosyan, 1999). On the contrary, in case of
Rosmarinus officinalis, the foliar application of Fe-DTPA did not show marketable increase in essential oil content (Moretti and Peana, 1998). Addition of Fe-EDTA to the nutrient solution of peppermint in hydroponic culture resulted in essential oil decrease (Mairapetyan Tadevosyan, 1999).

The present study deals with iron nutrition of Greek oregano [Origanum vulgare spp. hirtum (Link) Ietswaart], a plant of high economic value, which already is being cultivated commercially in the Mediterranean area. The effect of iron on herbage yield and essential oil quantity of oregano plants grown in the solution culture is discussed.

MATERIALS AND METHODS

Experimental Lay-out

The experiment was conducted in an unheated greenhouse at the Subtropical Plant and Olive Trees Institute, Chania, Greece. Economakis (1989) previously described the NFT system used in trials. The experiment occupied three PVC channels. The cultivation period in NFT lasted from 04.12.2000 to 14.06.2001. Photosynthetically active radiation (PAR), air temperature and relative humidity were recorded over the growing period (Table 1).

A modified nutrient solution formulated for oregano in NFT culture (Fournarakis, 1998) was used. Three levels of iron, i.e. 2.5 mg/l (Fe1); 5.0 mg/l (Fe2) and 11.0 mg/l (Fe3), were the target values in the nutrient solution (Fig. 1). Approximately once per month the old nutrient solution was dumped and a fresh one was prepared.

The target pH and EC values of all the three nutrient solutions were 6 and 2 mS/cm, respectively, measured by portable pH and EC meters. Target pH and EC were controlled by daily addition of 5% HNO₃ and the required amount of corresponding stock solutions.

The plant material used consisted of single plant cuttings of Origanum vulgare spp. hirtum rooted in a peat-based rooting media under mist propagation and transferred to the NFT channels 50 cm apart.

Statistical analysis was done by SPSS.

Nutrient Solution and Tissue Analysis

Samples of nutrient solution were taken weekly and analyzed by an Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES), Leeman Labs Inc., Model PS 1000-AT. Analytical lines for each element described were chosen according to EPA 200.7 method. The nutrient uptake/plant curve for iron was plotted by using differences of concentration values for two subsequent measurements, summed up by the concentration of iron in the stock solution added for the referred week and dividing by the number of plants in the channel.

Leaves collected for tissue analysis were gathered from all plants and mixed together. Collected leaves were sorted into “top”, “middle” and “bottom” depending on their position on the stem. Two chemical tissue analyses were performed for each treatment.

Roots were thoroughly washed under tap water prior to analyzing.

Samples were placed in an oven at 45 °C for 1 week. After drying the tissue was homogenized and taken for ashing in a muffle furnace, which was slowly brought to 500 ° C and kept there for 5 hours. The remaining ash was dissolved in 10 ml 2 NHCl. The volume was brought to 50 ml by addition of distilled water. The tissue elemental content was determined by ICP.

Essential Oil Distillation

Shoots subject to essential oil analysis were taken separately from each individual plant and dried on a lab bench at ambient temperature. Stems were removed from the dry shoots and 10 g of the remaining leafy fraction was taken for essential oil determination.
RESULTS

A considerable fluctuation of the target Fe nutrient levels was found in the solution. Readings were close to the starter concentration only on the days when a new solution was prepared, and tended to decrease during subsequent weeks, this phenomenon was more prominent when the plants entered flowering and seed formation phases. The average actual iron concentrations in the nutrient solution within the whole growth period for Fe1, Fe2 and Fe3 treatments were 1.43, 3.78 and 8.04 mg/l, respectively.

During the vegetative stage, Fe uptake was more or less constant for all the 3 treatments (Fig. 2) fluctuating around the values of 4.71(Fe1), 9.11(Fe2) and 14.15 (Fe3) mg/plant/week with peaks corresponding to the days when new nutrient solution was prepared. From the bud initiation stage onwards the Fe uptake increased drastically for all treatments and reached its maximum at the stage of seed formation (Fig. 2).

Tissue (leaves and roots) iron content was generally associated with solution iron concentration (Table 2). The older (basal) leaves had higher tissue iron content values than the younger ones, but far lower than that of the roots (Table 2)

The effect of iron concentrations on fresh and dry mass of shoot as well as on dry mass of stem was inconsistent (Table 3), nevertheless, Fe2 treatment gave the highest values for the mentioned parameters in absolute numbers, being significantly higher than Fe3. The weight of dry leaves was not significantly different among treatments, though the Fe2 treatment again gave the highest values in absolute terms (Table 3). The dry leaves/stem ratio was the highest for Fe3 plants, while the dry mass of the root was the lowest in this treatment.

Statistical analysis showed that essential oil content was the lowest in Fe3 plants, while those of Fe1 and Fe2 plants were not significantly different at 0.05 confidence level (Table 4).

DISCUSSION

Iron uptake by plants was associated with the Fe concentration in the nutrient solution. Among the treatments, the uptake was the highest for Fe3, followed by Fe2 and the lowest for Fe1 plants. Also within each treatment the peaks in Fe uptake correspond to the weeks when new nutrient solution was prepared, and consequently, the concentration of iron in the nutrient solution was the highest. During the subsequent weeks Fe uptake tended to decrease following the pattern of iron concentration in the nutrient solution. From the bud initiation stage onwards the iron uptake increased and reached its maximum at the seed formation period. Hood et al (1993) observed similar pattern of iron uptake with Antirrhinum majus grown in solution culture. The authors reported an increase in mean uptake of iron from the vegetative stage to anthesis. Lasztity (1999) reported that in an experiment with millet (Panicum miliaceum) under field conditions the uptake of Fe reached its maximum at full ripeness phase. Environmental conditions, especially air relative humidity, are also reported to affect iron uptake. In a field experiment with green tea (Camellia sinensis) high relative humidity was shown to hinder iron uptake (Sud et al, 1995). According to the meteorological data (Table 1) the relative humidity in the greenhouse decreased starting from March, which is the period corresponding to increased Fe uptake.

Plants of treatment Fe1 were lighter in color and manifested symptoms of chlorosis, especially on young, newly developed leaves. The increase in tissue iron content with increasing solution Fe concentration (Table 3) agrees with the results reported by Misra (1993) in an experiment with Mentha arvensis in solution culture. However, in contrast to our results, the same author reported no effect of Fe accumulation in a Clevenger apparatus described in European Phramacopoeia. Each distillation lasted 3 hours. The content of each sample was expressed in ml of essential oil per 100 g of dry leaf material. The mean of the hydrodistillation of 6 experimental plants of each treatment was considered as a mean value of essential oil content for that treatment.
in leaves in relation to their position (age). In our results younger leaves had less Fe content than the old ones (Table 3).

Higher values for fresh and dry mass of shoot, dry mass of stem and root were recorded for Fe2 treatment, when 5 mg/l iron concentration was the target value in the nutrient solution. Similar results were observed for a solution culture of Japanese mint (*Mentha arvensis* L.), under solution Fe concentration varying from 0.0 to 22.4 mg/l, where the treatment with 5.6 mg/l EDTA gave the highest fresh and dry matter yield (Misra and Sharma, 1991). Similar to our results, an increase in tissue Fe content with increasing solution’s Fe concentration from 5 to 50 mg/l was reported by Mairapetyan and Tadevosyan (1999) in an experiment with citric *Sorghum* in gravel culture. The plants supplied with 5 mg/l Fe-EDTA had higher yield that the ones fed with 50 mg/l Fe-EDTA. Nevertheless, for the peppermint in gravel culture the same author reported that application of 5 mg/l, 10 mg/l or 15 mg/l of Fe-EDTA in the nutrient solution had no effect on shoot dry weight.

The essential oil content of leaves was significantly lower in plants of the Fe3 treatment, being in agreement with the findings of Mairapetyan and Tadevosyan (1999) for peppermint, where an increase of Fe concentration in the nutrient solution from 5 mg/l to 15 mg/l resulted in decrease of essential oil content by 17-27%. Similarly, Misra and Srivastava (1989) obtained lower values for the concentration of the essential oil of *Mentha arvensis* L., when the plants were grown in a nutrient solution having 11.2 mg/l Fe-EDTA compared with the treatment of 5.6 mg/l, and a further decrease in essential oil content for the treatment with 22.4 mg/l iron concentration.

It could be concluded that the highest iron concentration in the nutrient solution resulted in high iron uptake and high tissue (leaves and roots) iron content, retarding growth and essential oil production of oregano plants either directly (toxicity) or through induced deficiency of other nutrients.

**ACKNOWLEDGEMENTS**

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**Literature Cited**


**Tables**

Table 1. Meteorological conditions in the glasshouse during the cultivation period

<table>
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<tr>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Average air temperature, °C</td>
<td>14.1±3.2</td>
<td>14.±3.0</td>
<td>13.9±2.9</td>
<td>18.6±5.0</td>
<td>18.6±3.9</td>
<td>23.4±4.6</td>
<td>27.3±4.4</td>
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<tr>
<td>PAR, E/m²</td>
<td>63494</td>
<td>150145</td>
<td>259052</td>
<td>216720</td>
<td>347437</td>
<td>251311</td>
<td>49742*</td>
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<tr>
<td>Average relative humidity, %</td>
<td>95.4±10.2</td>
<td>95.2±10.8</td>
<td>90.3±10.8</td>
<td>74.0±23.1</td>
<td>71.8±18.8</td>
<td>78.6±19.1</td>
<td>68.7±16.7</td>
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*The glasshouse was whitewashed on 12.06.01
Table 2. Iron content of the leaves (top, middle, bottom) and roots of oregano plants sampled at flowering stage, for 3 iron treatments (mg/g dry leaf mass)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Leaves (mg/g dry leaf mass)</th>
<th>Roots (mg/g dry leaf mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Top</td>
<td>Middle</td>
</tr>
<tr>
<td>Fe1</td>
<td>0.095</td>
<td>0.104</td>
</tr>
<tr>
<td>Fe2</td>
<td>0.121</td>
<td>0.121</td>
</tr>
<tr>
<td>Fe3</td>
<td>0.125</td>
<td>0.120</td>
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<tr>
<td>Average</td>
<td>0.114</td>
<td>0.115</td>
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</tbody>
</table>

Table 3. The production parameters of oregano plants harvested at the flowering stage in three iron treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Shoot fresh mass (g/plant)</th>
<th>Shoot dry mass (g/plant)</th>
<th>Stem dry mass (g/plant)</th>
<th>Dry weight of leaves (g/plant)</th>
<th>Dry leaf/stem ratio</th>
<th>Root dry mass g/plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe1</td>
<td>675.0 ab</td>
<td>175.0 ab</td>
<td>94.6 ab</td>
<td>80.6 a</td>
<td>0.84 b</td>
<td>33.9 a</td>
</tr>
<tr>
<td>Fe2</td>
<td>837.0 a</td>
<td>231.0 a</td>
<td>127.0 a</td>
<td>104.0 a</td>
<td>0.83 b</td>
<td>37.7 a</td>
</tr>
<tr>
<td>Fe3</td>
<td>591.0 b</td>
<td>166.0 b</td>
<td>83.2 b</td>
<td>82.3 a</td>
<td>1.02 a</td>
<td>21.2 b</td>
</tr>
</tbody>
</table>

*Treatment means within columns followed by the same letter are not significantly different at 0.05 level

Table 4. Essential oil content (ml/100 g of dry leaves + inflorescence) of oregano plants in three iron treatments sampled in May at the anthesis.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Essential oil %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe1</td>
<td>7.48 a</td>
</tr>
<tr>
<td>Fe2</td>
<td>7.38 a</td>
</tr>
<tr>
<td>Fe3</td>
<td>6.74 b</td>
</tr>
</tbody>
</table>
Figures

Fig. 1. Fluctuations of iron concentration in the nutrient solution during the cultivation period (arrows show the days, when the nutrient solution was renewed. The corresponding growth phases: V—vegetative stage, B—bud formation, F—flowering, S—seed formation)

Fig. 2. Weekly iron uptake of oregano plants from a nutrient solution with 3 Fe levels (arrows show the days, when the nutrient solution was renewed. The corresponding growth phases V—vegetative stage, B—bud formation, F—flowering, S—seed formation)