Effect of mycorrhizal inoculation and phosphorus supply on morphological traits of rosemary under greenhouse conditions

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Abstract

Rhizobium inoculation increases nutrients uptake by modification of root characteristics. This experiment was conducted in 2015 at Zabol university research farm (Chah-Nimeh) in a completely randomized design based on factorial arrangement with three replications. The first factor was five levels of phosphate: 100, 75, 50, 25 and 0 (control) kg ha⁻¹ and the soil inoculation consisted of two arbuscular mycorrhizal: *G. mosseae* and *G. intraradices*. The measured traits include number of leaves, stem dry weight, root fresh weight, shoot dry weight, stem diameter, root length, plant height, SPAD readings, root and shoot nitrogen content, essential oil percentage and essential oil yield. Results indicated that using of *G. intraradices* and *G. mosseae* have no significant effects on rosemary essential oil yield. The highest and lowest essential oil percentage rate of 2.2 and 1.6 %, respectively were as a result of taking ammonium phosphate 100 kg ha⁻¹ and in the control (no ammonium phosphate). On the other hand, higher shoot (1.17) and root (1.96 ) nitrogen percentage and were recorded followed by interaction between *G. mosseae* species and the control, respectively. The SPAD readings of rosemary increased significantly by the application of fertilizer in levels. On interaction effects, *G. intraradices* (M1) and application of 75 kg ha⁻¹ ammonium phosphate treatments had the best SPAD readings. The results of this study indicated that the inoculation of arbuscular mycorrhizal fungi in soil with optimal fertilizer application greatly improved rosemary growth and nutrient uptake and the effect was greater under greenhouse conditions.

Key words: Essential oil, fertilizer, *Glomus*, root, shoot, SPAD value.

Introduction

Rosemary (*Rosmarinus officinalis* L.) has long been considered as an important plant because of its essential oil used in perfumes and medicine as well as an important spice and antioxidant in processed foods. It is also a delightful herb with ornamental value that may stretch beyond the herb garden, either being used as a standard Armitage, or as a holiday pot plant at Christmas (DeBaggio, 1987; Armitage 1997). For these reasons and other reasons, it has been grown since ancient times (Simon et al., 1984).

Even Shakespeare’s Ophelia pays tribute to rosemary in Hamlet. Rosemary is a member of the mint family, Lamiaceae. It has opposite, simple, entire, evergreen leaves up to two inches long and an eighth of an inch wide. The leaf margins are revolute and the leaves are a shiny green on top and whitish beneath due to a dense collection of very fine hairs (Dirr, 1990). The plant begins to flower in late winter and continues through spring (Armitage, 1997). Despite the plant popularity and its many uses, there is very limited published research on the growing criteria. (Boyle et al., 1991) found out that rosemary does not respond well to high levels of fertilizer, but the ideal fertilization concentration was not determined. For this reason, the global research focus is on the production of medicinal plants under sustainable farming systems through various management techniques. One of the most important management techniques is to increase the use of bio-fertilizers and reduce the chemical inputs in the soils, especially in arable land under cultivation of medicinal plants.

Mycorrhiza is a term coined to describe the interaction of soil fungi and plant roots. In general, these soil fungi evolved from the symbiotic association with plant roots. Both plants and fungi gained chemical, physical, biological, and physiological benefits. The management of this association showed an increase in agricultural and natural plant growth. Colonization of plant roots by arbuscular mycorrhizal fungi can greatly increase the plant uptake of phosphorus and nitrogen. The most prominent contribution of arbuscular mycorrhizal fungi to plant growth is as a result of the uptake of nutrients by extra radical mycorrhizal hyphae. Quantification of hyphal nutrient uptake has become possible by using soil boxes with separated growing zones for roots and hyphae. Many (but not all) tested fungal isolates increased phosphorus and nitrogen uptake of the plant by absorbing phosphate, ammonium, and nitrate from the soil. However, when compared with the nutrient demand of the plant for growth, the contribution of arbuscular mycorrhizal fungi to the plant phosphorus uptake is usually much larger than the contribution to the plant nitrogen uptake.

The two pathways by which arbuscular mycorrhizal plants absorb phosphorus involve different cell types, (1) different Pi transporters (PtTs) and (2) P access from different regions and volumes of the soil. Direct uptake by root epidermis (including root hairs when they are formed), accesses Pi in the soil solution close to the roots. Expression of genes encoding high-affinity PtTs in these cells are in its maximum in the root apex and root

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Mycorrhizal inoculation and phosphorus and rosemary on early establishment and morphological traits of Rosemary under greenhouse conditions.

Material and methods

The effects of mycorrhizae and phosphorus fertilizer on early establishment and morphological traits of Rosemary under greenhouse condition were studied at the Agricultural Research Institute, University of Zabol, Iran in 2015. This greenhouse is located at latitude 30° 54’ N, longitude 61° 41’ E, and an altitude of 481 m above mean sea level.

A completely randomized design was used based on factorial arrangement with three replications. The first factor consisted of five levels of phosphorus: 100, 75, 50, 25 and 0 (control) kg per ha was applied as ammonium phosphate fertilizer, and the second factor was the inoculation of soil with two mycorrhizae fungi species: G. intraradices (M1) and G. mosseae (M2). On the 5th of March, before planting Rosemary seedlings, chemical fertilizers were mixed with soil in pots. After fertilization, the seedlings were planted in each pot and then all pots were placed for 16 weeks in a greenhouse at temperature 30±2 °C and 65 % relative humidity. Urea was applied at 3 stages (early planted, early vegetative and early reproductive growth). Irrigation and weed control was carried out in the pots during the growing season. The SPAD value (SPAD 502 made by Minolta Co., Ltd.) representing green degree of a leaf was measured and the total nitrogen content of the rosemary was also determined by combustion N analysis and/or Kjeldahl N analysis (AOCS, 1990). Traits were measured, including number of leaves, stem dry weight, root fresh weight, shoot dry weight, shoot fresh weight, stem diameter, root length, plant height, SPAD readings, root nitrogen content, shoot nitrogen content, essential oil yield and essential oil percentage. Essential oil content in the plant was determined by laboratory distillation in a Clevenger’s apparatus. Moisture was determined by drying a weighed sample of the plant in an oven at 35°C for 48 hours and the dry matter (plant) was weighed. The dried samples were preserved for nutrient analysis. Oil yields were calculated from dry matter and oil content was calculated on a dry basis. Data were analyzed by analysis of variance (ANOVA) using SAS version 9 (SAS institute, Inc., Cary, N.C.).

Results and discussion

Plant height: The results of the analysis of variance showed that the impact of the mycorrhizae and fertilizer treatment and interaction between mycorrhizae and different levels of fertilizer on this trait was significantly different at 1% (Table 2). According to the compared data,
the highest 195.1 mm and lowest 110.1 mm plant height were obtained, respectively from *G. mosseae* and application of 100 kg ha⁻¹ ammonium phosphate treatment and *G. mosseae* and application of 0 kg ha⁻¹ ammonium phosphate treatment (Table 4). Researchers reported that with increasing in mycorrhizae fungi rate, the plant height of acacia (*Acacia holosericea* L.) also increased (Dupponnois et al., 2005). Mycorrhizal fungi are beneficial micro-organisms closely associated with the roots of most plants. These fungi enable plants to absorb more nutrients, such as phosphorus, zinc, and copper from many soils than the corresponding non-mycorrhizal plants. Mycorrhizal fungi may also increase water uptake. In this way, mycorrhizal fungi increase the efficiency of fertilization. Also, Ghorbanian et al. (2011) reported that mycorrhizal fungi, by extending their root absorbing area through their mycelium network and changing unavailable phosphorus to available form and translocate to root system cause increase in plant height and growth parameters. The highest values in the plant heights of *Zea mays* were observed when *G. mosseae* was used (Abdelmonem et al., 2014). Martinetti et al. (2003) mentioned that the highest plant height of rosemary was observed when 200 mg plant⁻¹ N, 40 mg plant⁻¹ P₂O₅ and 200 mg plant⁻¹ K₂O were combined. The results of Rahimi et al. (2016) suggested that partial replacement of phosphorus chemical fertilizers by biological sources increased the flower yield of *Calendula officinalis* L. using both biofertilizers (*Glomus intraradices, G. mosseae* and *G. hoi*) and chemical P.

**Root length:** Analysis of variance showed that the effect of mycorrhizae and different levels of fertilizer treatments on root length was significant at 1% (Table 2) and the root length did not vary due to the interaction between mycorrhizae and different levels of fertilizer. Also, the use of more fertilizer increased the root length, and this increase was significant. The highest root length 174.9 mm was obtained from using 100 kg ha⁻¹ ammonium phosphate (control) was not applied (Table 3). The recorded higher root length was 144.1 mm, followed by *G. intraradices* species 126 mm and the least recorded root length in *G. mosseae* species was 96.4 mm (Table 3). The results of Bhartia et al. (2016) showed that the bio-inoculants (*Dietzia natronolimnaea* and *G. intraradices*) improved *Ocimum basilicum* growth under salinity stress in both glasshouse and field conditions.

**Stem diameter:** The effect of experimental treatments on this trait was not significant (Table 2). It seems that in rosemary, the stem diameter is more influenced by the plant genetics. Interaction between mycorrhizae fungi and different levels of fertilizer treatments on the stem diameter was not significant (Table 2). The addition of nitrogen, phosphorus and potassium (NPK) treatments increased the leaf area, stem diameter, number of leaves, fresh weight and dry weight of *Cucurbita moschata* and all the NPK treatments had significantly broader leaf area, stem diameter, number of leaves, fresh and dry weight than the control (Okonwu and Mensah, 2012).

**Fresh and dry weight of shoot:** The results of this research showed that the fresh and dry weight of shoot was not affected by mycorrhizae species (Table 2). The effect of different levels of fertilizer treatment on these traits was significant at 5 and 1%, respectively (Table 2) and the use of more fertilizer increased the fresh and dry weight of the shoot. There was a significant difference between control (without ammonium phosphate) and first level of fertilizer 100 kg ha⁻¹. Higher shoot dry weight 8.04 g was obtained by using 75 kg ha⁻¹ fertilizer and the highest shoot fresh weight 8.6 g was in 75 kg ha⁻¹ fertilizer treatment. No significant difference was observed between the application of 100 kg ha⁻¹ ammonium phosphate treatment and the second level of fertilizer 75 kg ha⁻¹. Also, the lowest shoot fresh weight was obtained without applying fertilizer (control) (Table 3). The effect of the interaction between mycorrhizae species and different levels of fertilizer treatments on the fresh and dry weight of shoot was not significant (Table 2). Martinetti et al. (2003) reported that the highest fresh yield on rosemary was obtained by applying the combination of 200 mg plant⁻¹ N and 40 mg plant⁻¹ P₂O₅. Researchers reported the highest and lowest levels of shoot dry weight of wheat (*Triticum spp.*) under greenhouse conditions in bio-phosphor and control, respectively (Forouzandeh et al., 2014).

**Root fresh weight:** Regarding this trait, the effect of fertilizer treatment and interaction between mycorrhizae and different levels of fertilizer was significant (*P*=0.01) (Table 2). Based on the comparison of the means, the highest fresh root weight 1.59 g was obtained as a result of first level of ammonium phosphate fertilizer 100 kg ha⁻¹ with *G. mosseae*, and the lowest fresh root weight 0.21 g was obtained as a result of the control treatment 0 kg ha⁻¹ with *G. mosseae* (Table 4). This data is in agreement with the results of (Rezvani et al., 2010) in *Medicago sativa* L. Habibzadeh (2015) reported that colonization percentage of *G. mosseae* was more than *G. intraradices* and was less reduced with increasing phosphorus levels.

**Stem dry weight:** The effects of treatments on this trait were significant at 1% (Table 2). The interaction effect between Table 3. Mean of some characteristics of rosemary affected by Mycorrhiza and fertilizers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Root length (mm)</th>
<th>Stem diameter (mm)</th>
<th>Shoot fresh weight (g)</th>
<th>Shoot dry weight (g)</th>
<th>Root fresh weight (g)</th>
<th>Essential oil (%)</th>
<th>Essential oil yield (mg per pot)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>G. intraradices</em></td>
<td>144.1A</td>
<td>1.83A</td>
<td>7.83A</td>
<td>6.90A</td>
<td>0.73A</td>
<td>1.9A</td>
<td>1.01A</td>
</tr>
<tr>
<td><em>G. mosseae</em></td>
<td>126.0B</td>
<td>1.92A</td>
<td>8.00A</td>
<td>7.20A</td>
<td>0.76A</td>
<td>1.9A</td>
<td>1.07A</td>
</tr>
<tr>
<td>Fertilizer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>174.90A</td>
<td>2.06A</td>
<td>8.22A</td>
<td>7.53AB</td>
<td>1.40A</td>
<td>2.25A</td>
<td>1.09A</td>
</tr>
<tr>
<td>75</td>
<td>138.35BC</td>
<td>1.91AB</td>
<td>8.59A</td>
<td>8.04A</td>
<td>0.70B</td>
<td>2.21A</td>
<td>1.14A</td>
</tr>
<tr>
<td>50</td>
<td>147.65B</td>
<td>1.88AB</td>
<td>7.94AB</td>
<td>7.02BC</td>
<td>0.66B</td>
<td>2.02AB</td>
<td>1.09A</td>
</tr>
<tr>
<td>25</td>
<td>125.61C</td>
<td>1.73B</td>
<td>7.63AB</td>
<td>6.38C</td>
<td>0.61B</td>
<td>1.71B</td>
<td>1.03A</td>
</tr>
<tr>
<td>0</td>
<td>88.95D</td>
<td>1.78B</td>
<td>7.20B</td>
<td>6.27C</td>
<td>0.34C</td>
<td>1.68B</td>
<td>0.85B</td>
</tr>
</tbody>
</table>

Means followed by similar letters in each column are not significantly different at *P*=0.05, GLM multiple ranges test
Mycorrhizal inoculation and phosphorus and rosemary

Number of leaves per plant: Analysis of variance showed that the number of leaves per plant was significantly affected at 1 % level by mycorrhizae fungi, fertilizers and M×F treatments (Table 2). Interaction effects show that the G. mosseae species at 100 kg ha⁻¹ fertilizer treatment has the highest number of leaves per plant (45) among other treatments (Table 4). This shows that higher ammonium phosphate rates enhanced the vegetative growth of the rosemary and increased the source capacity of the plants by the number of leaves produced per plant. Nitrogen and phosphorus are the two elements that have positive effect on uptake and impact of biochemical interplay. In other word, by increasing nitrogen, phosphorus uptake by plant and its effect on plant metabolic activity increases and with increasing soil phosphorus, nitrogen uptake and physiological effects of the plant increases. Phosphorus that is a major factor in the storage and transfer of energy within the plant can be used as a major factor in the stored energy for the metabolic processes of the plant (Mahmoudi and Hakimian, 2000). Also, the root system of plants inoculated with AMF were often more finely divided and thus have more absorptive surface area (Okon et al., 1996). Pal et al. (2016) showed that N, P and K fertilizations positively affect the development of Thymus and the highest yield was obtained in the highest rate of N (150 kg urea ha⁻¹), P (250 kg phosphorus ha⁻¹) and K (150 kg potash ha⁻¹) fertilizers.

Essential oil percentage: The results of this research indicated that the effect of the fertilizer levels on essential oil percentage was not significant (P<0.01) and the effect of mycorrhizae type and interaction M×F on essential oil yield was not significant (Table 2). The highest (1.14 mg pot⁻¹) and the lowest (0.85 mg pot⁻¹) essential oil yield was obtained in ammonium phosphate (75 kg ha⁻¹) and control (no ammonium phosphate), respectively. Comparing the mean values showed no significant difference between first 100 kg ha⁻¹, second 75 kg ha⁻¹, third 50 kg ha⁻¹ and fourth 25 kg ha⁻¹ levels of fertilizer (Table 3). The results of Bahonar et al. (2016) showed that the inoculation of mycorrhiza fungi (G. intraradices) improved the morphological and phytochemical traits of rosemary positively under different levels of salinity. Essential oil is a terpenoid compound and its components (isoprenoids) such as Isopantyl pyrophosphate (IPP) and Dimethyl Alyl pyrophosphate (DMAPP) highly demand NADPH, ATP, and the fertilizers such as nitrogen and phosphorous are required for production of the secondary compounds (Ghazi Manas et al., 2013). Ozguven et al. (2008) concluded that by increasing level of nitrogen fertilizer, essential oil content of Artemisia (Artemisia annua L.) increased.

Shoot and root nitrogen content: Regarding these traits, the effect of the mycorrhizae and fertilizer treatment was significant at 1 % level, but the interaction between mycorrhizae and different levels of fertilizer on shoot and root nitrogen content was significant at 1 and 5 %, respectively (Table 2). The highest shoot (1.17 %) and root (1.96 %) nitrogen was recorded in interaction of ammonia phosphates (75 kg ha⁻¹) and control (no ammonium phosphate), respectively. Comparing the mean values showed no significant difference between 100 kg ha⁻¹, 75 kg ha⁻¹, 50 kg ha⁻¹, 25 kg ha⁻¹ and control (no ammonium phosphate) treatments (Table 4). Soleymani and Pirzad (2016) showed the order of nitrogen uptake with mean 10.1 g kg⁻¹ dry matter in rosemary was obtained by the application of 80 mg plant⁻¹ P₂O₅.

Results of Soleymani and Pirzad (2016) showed the order

Table 4. Interaction between Mycorrhizae and fertilizer on some characteristics of rosemary

<table>
<thead>
<tr>
<th>Mycorrhizae</th>
<th>Fertilizer</th>
<th>Plant height (mm)</th>
<th>Root fresh weight (g)</th>
<th>Stem dry weight (g)</th>
<th>Number of leaves per plant</th>
<th>Nitrogen content</th>
<th>SPAD readings</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1 100</td>
<td>100</td>
<td>147.02BC</td>
<td>1.21B</td>
<td>1.37B</td>
<td>37B</td>
<td>0.38F</td>
<td>61.16B</td>
</tr>
<tr>
<td>75</td>
<td>124.96DC</td>
<td>0.71C</td>
<td>0.92DC</td>
<td>36B</td>
<td>0.42F</td>
<td>0.95E</td>
<td>78.83A</td>
</tr>
<tr>
<td>50</td>
<td>121.66D</td>
<td>0.59DC</td>
<td>1.29B</td>
<td>25.33C</td>
<td>0.47DF</td>
<td>0.91E</td>
<td>53.53BC</td>
</tr>
<tr>
<td>25</td>
<td>127.79DC</td>
<td>0.65C</td>
<td>0.71E</td>
<td>37B</td>
<td>0.58DCE</td>
<td>1.24D</td>
<td>48.43BC</td>
</tr>
<tr>
<td>0</td>
<td>129.54DC</td>
<td>0.48D</td>
<td>0.83DE</td>
<td>13.66D</td>
<td>0.64DC</td>
<td>1.50BC</td>
<td>52.30BC</td>
</tr>
<tr>
<td>M2 100</td>
<td>195.10A</td>
<td>1.59A</td>
<td>1.65A</td>
<td>45A</td>
<td>0.64DC</td>
<td>1.63B</td>
<td>57.10B</td>
</tr>
<tr>
<td>75</td>
<td>159.21B</td>
<td>0.69C</td>
<td>1.81A</td>
<td>35.66B</td>
<td>0.67C</td>
<td>1.37CD</td>
<td>57.70B</td>
</tr>
<tr>
<td>50</td>
<td>130.08DC</td>
<td>0.62DC</td>
<td>0.76DE</td>
<td>40AB</td>
<td>0.86B</td>
<td>1.56BC</td>
<td>53.46BC</td>
</tr>
<tr>
<td>25</td>
<td>122.89DC</td>
<td>0.68C</td>
<td>0.72E</td>
<td>29C</td>
<td>0.95B</td>
<td>1.59B</td>
<td>42.93C</td>
</tr>
<tr>
<td>0</td>
<td>110.17D</td>
<td>0.21E</td>
<td>1.01C</td>
<td>24.66C</td>
<td>1.17A</td>
<td>1.96A</td>
<td>58.23B</td>
</tr>
</tbody>
</table>

Means followed by similar letters in each column are not significantly different at P=5 %, GLM multiple ranges test
of highest colonization of Hyssop root was *G. mosseae*, *G. intraradices*, *G. fasciculatum*, *G. claroideum*, respectively. Also, any increase in the ammonium phosphate levels would reduce shoot and root nutrient content.

**SPAD readings:** The SPAD value of rosemary was affected by mycorrhizae and interaction of M×F at P=0.01 levels (Table 2). On interaction effects, *G. intraradices* (M1) and the application of 75 kg ha\(^{-1}\) ammonium phosphate treatment had the best SPAD value (Table 4). In addition, Habibzadeh and Abedi (2014) reported that inoculation of mung bean with *G. intraradices* and *G. mosseae* had no effect on the SPAD value. The effect of arbuscular mycorrhiza on the SPAD value in this experiment is similar to the results reported by other researchers (Mathur and Vyas, 2000; Srivastava et al., 2002).

The findings of the present study suggested that mycorrhizal fungi significantly increase plant growth and yield of rosemary, and could be replaced for chemical fertilizer. Application of both phosphorous and mycorrhizae could affect growth characteristics. In general, overall results indicated that positive impact of mycorrhizal symbiosis on rosemary was not related to fungi species and it seems phosphorous application at 75 kg ha\(^{-1}\) would be appropriate for rosemary production.

**References**


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